

JC11 Rec'd PCT/PTO SEP 19 2001

FORM PTO-1390 (Modified)
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

110.01270101

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/937076

INTERNATIONAL APPLICATION NO.
PCT/US00/07680

INTERNATIONAL FILING DATE
22 March 2000

PRIORITY DATE CLAIMED
22 March 1999

TITLE OF INVENTION

METHODS OF USE OF BETA1-INTEGRIN INHIBITORS

APPLICANT(S) FOR DO/EO/US

James B. McCarthy, William J. Mileski, Gordon A. Jamieson, Jr., Walter C. Low, Ronald J. Sawchuk, and Leo T. Furcht

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Formal Drawings (16 sheets)

| | | | | | |
|---|--------------|-------------------------------|--------------------------|---------------------------|----|
| U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.53(a)(2)) | | INTERNATIONAL APPLICATION NO. | | ATTORNEY'S DOCKET NUMBER | |
| 09/937076 | | PCT/US00/07680 | | 110.01270101 | |
| 24. The following fees are submitted: | | | | CALCULATIONS PTO USE ONLY | |
| BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : | | | | | |
| <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO | | | | \$1000.00 | |
| <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO | | | | \$860.00 | |
| <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO | | | | \$710.00 | |
| <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) | | | | \$690.00 | |
| <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) | | | | \$100.00 | |
| ENTER APPROPRIATE BASIC FEE AMOUNT = | | | | \$860.00 | |
| Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 | | | | \$130.00 | |
| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | |
| Total claims | 40 - 20 = | 20 | x \$18.00 | \$360.00 | |
| Independent claims | 9 - 3 = | 6 | x \$80.00 | \$480.00 | |
| Multiple Dependent Claims (check if applicable). | | | <input type="checkbox"/> | \$0.00 | |
| TOTAL OF ABOVE CALCULATIONS = | | | | \$1,830.00 | |
| <input checked="" type="checkbox"/> Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2. | | | | \$915.00 | |
| SUBTOTAL = | | | | \$915.00 | |
| Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30 | | | | \$0.00 | |
| TOTAL NATIONAL FEE = | | | | \$915.00 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). | | | <input type="checkbox"/> | \$0.00 | |
| TOTAL FEES ENCLOSED = | | | | \$915.00 | |
| | | | | Amount to be: refunded | \$ |
| | | | | charged | \$ |
| a. <input checked="" type="checkbox"/> A check in the amount of \$915.00 to cover the above fees is enclosed. | | | | | |
| b. <input type="checkbox"/> Please charge my Deposit Account No. in the amount of to cover the above fees. A duplicate copy of this sheet is enclosed. | | | | | |
| c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-4895 A duplicate copy of this sheet is enclosed. | | | | | |
| d. <input type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. | | | | | |
| NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status. | | | | | |
| SEND ALL CORRESPONDENCE TO: | | | | | |
| Ann M. Mueting Mueting, Raasch & Gebhardt, P.A. P.O. Box 581415 Minneapolis, Minnesota 55458-1415 United States of America | | | | | |
| SIGNATURE | | | | | |
| Ann M. Mueting | | | | | |
| NAME | | | | | |
| 33,977 | | | | | |
| REGISTRATION NUMBER | | | | | |
| 19 September 2001 | | | | | |
| DATE | | | | | |

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): James B. McCarthy, et al.

Group Art Unit: 1642

Serial No.: 09/937,076

Examiner: Unknown

Filed: September 19, 2001

Docket No.: 110.01270101

Int'l Filing Date: March 22, 2000

Confirmation No.: 4527

Title: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

Assistant Commissioner for Patents
BOX PCT
Washington, D.C. 20231

We are transmitting the following documents along with this Transmittal Sheet (which is submitted in triplicate):

- ☒ **Small entity status is entitled to be asserted in the above-identified application.**
- ☒ An itemized return postcard.
- ☒ A Petition for Extension of Time for 2 month(s) and a check in the amount of \$200 for the required fee.
- ☐ An Information Disclosure Statement (pgs), copies of applications; 1449 forms (pgs); and copies of documents cited on the 1449 forms.
- ☐ A check in the amount of \$, representing .
- ☒ Other: Response to Notification of Missing Requirements (1 pg) and copy of Notification of Missing Requirements (2 pgs); Signed Declaration and Power of Attorney (8 pgs).
- Amendment No Additional fee is required. The fee has been calculated as shown:

| Fee Calculation for Claims Pending After Amendment | | | | | |
|---|------------------------------------|-----------------------------|-----------------------------------|---------------------------|--------------------------|
| | Pending Claims after Amendment (1) | Claims Paid for Earlier (2) | Number of Additional Claims (1-2) | Cost per Additional Claim | Additional Fees Required |
| Total Claims | | | | x \$9 = | |
| Independent Claims | | | | x \$42 = | |
| One or More New Multiple Dependent Claims Presented? If Yes, Add \$140 Here → | | | | | |
| Total Additional Claim Fees Required | | | | | |

Please consider this a PETITION FOR EXTENSION OF TIME for a sufficient number of months to enter these papers and please charge any additional fees or credit overpayment to Deposit Account No. 13-4895. Triplicate copies of this sheet are enclosed.

MUETING, RAASCH & GEBHARDT, P.A.

Customer Number: 26813



26813

PATENT TRADEMARK OFFICE

By: Ann M. Mueeting
Name: Ann M. Mueeting
Reg. No.: 33,977
Direct Dial: 612-305-1217
Facsimile: 612-305-1228

CERTIFICATE UNDER 37 CFR §1.10:

"Express Mail" mailing label number: EL 888270523 US

Date of Deposit: March 27, 2002

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, BOX PCT, Washington, D.C. 20231.

By: Gara L. Ladwig
Name: Gara L. Ladwig

(SMALL ENTITY TRANSMITTAL UNDER RULE 1.10)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): James B. McCarthy et al.)
Serial No.: 09/937,076)
Confirmation No.: 4527)
Filed: September 19, 2001)
International Filing Date: March 22, 2000)
Group Art Unit: 1642
Examiner: Unknown
For: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents
BOX PCT
Washington, DC 20231

In accordance with the provisions of 37 C.F.R. §1.136(a), it is respectfully requested that a two-month extension of time be granted in which to respond to the Notification of Missing Requirements mailed November 27, 2001, thereby extending the date on which the period of response is set to expire from January 27, 2002, to March 27, 2002.

Our check in the amount of \$200.00 is enclosed to pay the required extension fee. Please charge any additional fees or credit any over-payment to PTO Deposit Account No. 13-4895.

CERTIFICATE UNDER 37 C.F.R. 1.10:

The undersigned hereby certifies that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated below and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

Gara L. Ladwig
Gara L. Ladwig
"Express Mail" mailing label number:
EL 888270523 US
Date of Deposit: 27 March 2002

March 27, 2002
Date

Respectfully submitted for
James B. McCarthy, et al.
By Muetting, Raasch & Gebhardt, P.A.
P.O. Box 581415
Minneapolis, MN 55458-1415
Phone: (612)305-1220
Facsimile: (612)305-1228
Customer Number 26813



26813

PATENT TRADEMARK OFFICE

By: *Ann M. Muetting*

Ann M. Muetting
Reg. No. 33,977
Direct Dial (612)305-1217

PATENT
Attorney Docket No. 110.01270101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | |
|---|---|-----------------|---------|
| Applicant(s): James B. McCarthy et al. |) | Group Art Unit: | 1642 |
| |) | | |
| Serial No.: 09/937,076 |) | Examiner: | Unknown |
| Confirmation No.: 4527 |) | | |
| |) | | |
| Filed: September 19, 2001 |) | | |
| International Filing Date: March 22, 2000 |) | | |

For: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

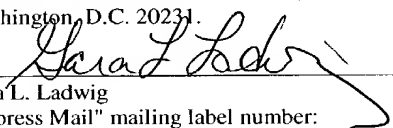
RESPONSE TO NOTIFICATION OF MISSING REQUIREMENTS

Assistant Commissioner for Patents
BOX PCT
Washington, D.C. 20231

In response to the "Notification of Missing Requirements Under 35 U.S.C. 371" (copy enclosed), an executed Declaration by the named inventors is enclosed. The surcharge for providing the Declaration later than the appropriate 20 or 30 months from the priority date was previously paid. The application should now be in condition for examination. Please direct any inquiries to the undersigned attorney.

CERTIFICATE UNDER 37 C.F.R. 1.10:

The undersigned hereby certifies that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated below and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.


Gara L. Ladwig
"Express Mail" mailing label number:
EL 888270523 US
Date of Deposit: 27 March 2002

March 27, 2002
Date
AMM/mi

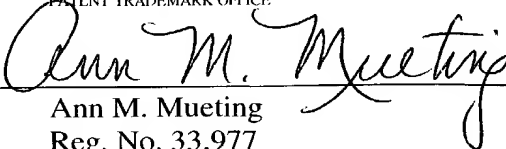
Respectfully submitted for
James B. McCarthy, et al.
By Mueting, Raasch & Gebhardt, P.A.
P.O. Box 581415
Minneapolis, MN 55458-1415
Phone: (612)305-1220
Facsimile: (612)305-1228
Customer Number 26813



26813

PATENT TRADEMARK OFFICE

By:


Ann M. Mueting
Reg. No. 33,977
Direct Dial (612)305-1217



UNITED STATES PATENT AND TRADEMARK OFFICE

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 Department of Commerce
 Washington, D.C. 20590
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| | | |
|----------------------------|-----------------------|----------------|
| U.S. APPLICATION NUMBER NO | FIRST NAMED APPLICANT | ATTY DOCKET NO |
| 09/937,076 | James B McCarthy | 110 01270101 |

26813
 MUETING, RAASCH & GEBHARDT, P.A.
 P.O. BOX 581415
 MINNEAPOLIS, MN 55458

RECEIVED

DEC 06 2001

MUETING & RAASCH

| | |
|------------------------------|---------------|
| INTERNATIONAL APPLICATION NO | |
| PCT/US00/07680 | |
| 1A FILING DATE | PRIORITY DATE |
| 03/22/2000 | 03/22/1999 |

CONFIRMATION NO. 4527

371 FORMALITIES LETTER



OC000000007118384

Date Mailed: 11/27/2001

NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as an Elected Office (37 CFR 1.495):

- U.S. Basic National Fees
- Indication of Small Entity Status
- Priority Document
- Copy of IPE Report
- Copy of references cited in ISR
- Copy of the International Application
- Copy of the International Search Report
- Preliminary Amendments
- Request for Immediate Examination
- Substitute Specification

MParts due 1/27/02
 MParts Stat 5/27/02

The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.

ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTH FROM THE DATE OF THIS NOTICE OR BY 22 or 32 MONTHS (where 37 CFR 1.495 applies) FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed

to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

*A copy of this notice **MUST** be returned with the response.*

MAMIE P PERSON

Telephone: (703) 305-3737

PART 1 - ATTORNEY/APPLICANT COPY

| U.S. APPLICATION NUMBER NO | INTERNATIONAL APPLICATION NO | ATTY DOCKET NO |
|----------------------------|------------------------------|----------------|
| 09/937,076 | PCT/US00/07680 | 110 01270101 |



09/937,076
PTO/PCT Rec'd 19 MAR 2002

PATENT
Attorney Docket No. 110.01270101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | |
|---|---|-----------------|---------|
| Applicant(s): James B. McCarthy et al. |) | Group Art Unit: | 1642 |
| |) | | |
| Serial No.: 09/937,076 |) | Examiner: | unknown |
| Confirmation No.: 4527 |) | | |
| |) | | |
| Filed: September 19, 2001 |) | | |
| International Filing Date: March 22, 2000 |) | | |

For: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to taking up the above-identified application for examination, please amend the application as follows:

Please replace the paragraph beginning at page 35, line 2, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Transient cerebral ischemia and associated brain injury may be mediated by several factors, including inflammatory processes (Hallenbeck et al., Stroke, 17, 246-253 (1986)). Leukocyte infiltration into ischemic tissue is a pathophysiological response, which often further aggravates ischemic injury by attenuating microvascular blood flow, and releasing chemical mediators such as free oxygen radicals (Kochanek et al., Stroke, 23, 1367-1379 (1992); and Matsuo et al., J. Cereb. Blood Flow Met., 15, 941-947 (1995)). Cell adhesion molecules play important roles in leukocyte-endothelial interactions: the selectins (Lasky, Science, 258, 964-969 (1992)), the integrins, and the immunoglobulin superfamilies (Springer, Nature, 346, 425-434 (1990)). Integrins which contain β_1 subunits usually are associated with mediating adhesion to extracellular matrix

Preliminary Amendment

Page 2

Applicant(s): James B. McCarthy et al.

Serial No.: 09/937,076

Confirmation No.: 4527

Filed: September 19, 2001

International Filing Date: March 22, 2000

For: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

constituents (Springer, Nature, 346, 425-434 (1990)) whereas β_2 integrins are largely involved in cell-cell interactions. One of these extracellular matrix macromolecules is fibronectin, which is found in plasma, cell matrix, and on the cell surface. These molecules can support leukocyte adhesion to endothelial cells (Akiyama et al., Adv. Enzymol., 59, 1-57 (1987)).

Please replace the paragraph beginning at page 35, line 18, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Fibronectin possesses multiple domains recognized by integrins, including arginyl-glycyl-aspartic acid (RGD). The latter interacts selectively with $\alpha 5\beta 1$ integrin, and the alternately spliced connecting segment domain (CS-1) which is recognized selectively by $\alpha 4\beta 1$ integrin (Akiyama et al., Adv. Enzymol., 59, 1-57 (1987); and Guan et al., Cell, 60, 53-61 (1990)). Over the last few years several novel (nonRGD/nonCS-1) bioactive peptides from fibronectin that: a) antagonize leukocyte adhesion of activated lymphocytes and monocytes *in vitro* when used as soluble antagonists and b) show efficacy for improved outcomes in several *in vivo* animal models of chronic and acute inflammation when administered intravenously. These models include bacterial cell wall-induced arthritis in rats, models of autoimmune disease such as TGF- β -/- mice, and reperfusion injury in rat transient cerebral ischemia and in rabbit burn models (Hines et al., Proc. Natl. Acad. Sci., USA, 91, 5187-5191 (1994); Wahl et al., J. Clin. Invest., 94, 655-662 (1994); and unpublished data).

Preliminary Amendment

Page 3

Applicant(s): James B. McCarthy et al.

Serial No.: 09/937,076

Confirmation No.: 4527

Filed: September 19, 2001

International Filing Date: March 22, 2000

For: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

REMARKS

Please enter into the record the preliminary amendment described herein prior to examination and consideration of the above-identified application.

The above amendments are made to correct typographical errors in the specification and do not add new matter.

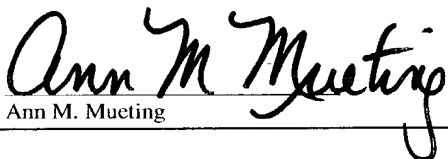
The amendments made to the article cited at page 35, line 17 and at line 21, were made to correct the volume number of the citation. The author, journal title, page number and year of publication were all cited correctly, and from this information the correct volume number may be easily found.

Conclusion

If the Examiner wishes to discuss any issues concerning this communication by telephone, please contact the below-signed attorney.

CERTIFICATE UNDER 37 C.F.R. 1.8:

The undersigned hereby certifies that this paper is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this 11 day of March, 2002.


Ann M. Muetting

March 11, 2002
Date

Respectfully submitted for

JAMES B. McCARTHY ET AL.

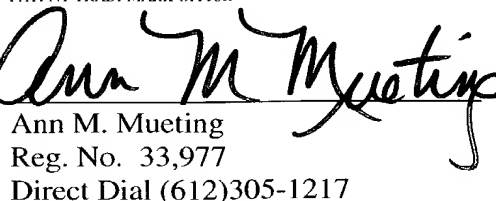
By
Muetting, Raasch & Gebhardt, P.A.
P.O. Box 581415
Minneapolis, MN 55458-1415
Phone: (612)305-1220
Facsimile: (612)305-1228
Customer Number 26813

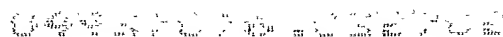


26813

PATENT TRADE-MARK OFFICE

By:


Ann M. Muetting
Reg. No. 33,977
Direct Dial (612)305-1217



For: METHODS OF USE OF $\beta 1$ -INTEGRIN INHIBITORS

Transient cerebral ischemia and associated brain injury may be mediated by several factors, including inflammatory processes (Hallenbeck et al., Stroke, 17, 246-253 (1986)). Leukocyte infiltration into ischemic tissue is a pathophysiological response, which often further aggravates ischemic injury by attenuating microvascular blood flow, and releasing chemical mediators such as free oxygen radicals (Kochanek et al., Stroke, 23, 1367-1379 (1992); and Matsuo et al., J. Cereb. Blood Flow Met., 15, 941-947 (1995)). Cell adhesion molecules play important roles in leukocyte-endothelial interactions: the selectins (Lasky, Science, 258, 964-969 (1992)), the integrins, and the immunoglobulin superfamilies (Springer, Nature, 346, 425-434 (1990)). Integrins which contain β_1 subunits usually are associated with mediating adhesion to extracellular matrix constituents (Springer, Nature, 346, 425-434 (1990)) whereas β_2 integrins are largely involved in cell-cell interactions. One of these extracellular matrix macromolecules is fibronectin, which is found in plasma, cell matrix, and on the cell surface. These molecules can support leukocyte adhesion to endothelial cells (Akiyama et al., Adv. Enzymol., [57]59, 1-57 (1987)).

**APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE**

Page A2

Applicant(s): James B. McCarthy et al.
Serial No.: 09/937,076
Confirmation No.: 4527
Filed: September 19, 1991
International Filing Date: March 22, 2000
For: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

The paragraph beginning at page 35, line 18, has been amended as follows:

Fibronectin possesses multiple domains recognized by integrins, including arginyl-glycyl-aspartic acid (RGD). The latter interacts selectively with α 5 β 1 integrin, and the alternately spliced connecting segment domain (CS-1) which is recognized selectively by α 4 β 1 integrin (Akiyama et al., Adv. Enzymol., ~~57~~⁵⁹, 1-57 (1987); and Guan et al., Cell, 60, 53-61 (1990)). Over the last few years several novel (nonRGD/nonCS-1) bioactive peptides from fibronectin that: a) antagonize leukocyte adhesion of activated lymphocytes and monocytes *in vitro* when used as soluble antagonists and b) show efficacy for improved outcomes in several *in vivo* animal models of chronic and acute inflammation when administered intravenously. These models include bacterial cell wall-induced arthritis in rats, models of autoimmune disease such as TGF- β $-/-$ mice, and reperfusion injury in rat transient cerebral ischemia and in rabbit burn models (Hines et al., Proc. Natl. Acad. Sci., USA, 91, 5187-5191 (1994); Wahl et al., J. Clin. Invest., 94, 655-662 (1994); and unpublished data).

PATENT
Attorney Docket No. 110.01270101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): James B. McCarthy, et al.)
Serial No.: Unknown)
Filed: Concurrently Herewith)
For: METHODS OF USE OF β 1-INTEGRIN INHIBITORS)

PRELIMINARY AMENDMENT

BOX PCT

Assistant Commissioner for Patents
Washington, D.C. 20231

Prior to examination and consideration of the above-identified U.S. national phase patent application, Applicants respectfully request entry of the annex to the International Preliminary Examination Report (PCT Article 34 amendments to the description, claims, and drawings), a copy of which is submitted herewith.

In addition to the above, Applicants respectfully request entry of the following amendment to the application:

IN THE SPECIFICATION

Please replace the paragraph beginning at page 1, line 7, under the heading, "Statement of Related Applications," with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

-- The present application is a national stage filing of International Patent Application No. PCT/US00/07680, filed on March 22, 2000, which in turn claims priority to U.S. Provisional Application Serial No. 60/125,634, filed March 22, 1999, and U.S. Provisional Application Serial No. 60/167,538, filed November 24, 1999, all of which are incorporated herein by reference. --

Preliminary Amendment

Page 2

Inventors : James B. McCarthy, et al.

Attorney Docket No.: 110.01270101

Title: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

REMARKS

This preliminary amendment requests entry of the Article 34 amendments made in the international application and which make up the annexes to the International Preliminary Examination Report. This amendment also updates the statement regarding related applications to include reference to the international application.

If the Examiner wishes to discuss any issues concerning this communication by telephone, please contact the below-signed attorney.

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APPENDIX A
SPECIFICATION / CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE

Applicant(s): James B. McCarthy, et al.
Serial No. Unknown
Filed Even Date Herewith
METHODS OF USE OF β 1-INTEGRIN INHIBITORS
Attorney Docket No. 110.01270101

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been bolded.

In the Specification

The paragraph beginning at page 1, line 7, has been amended as follows:

-- The present [invention] **application is a national stage filing of International Patent Application No. PCT/US00/07680, filed on March 22, 2000, which in turn** claims priority to U.S. Provisional Application Serial No. 60/125,634, filed March 22, 1999, and U.S. Provisional Application Serial No. 60/167,538, filed November 24, 1999, [both] **all** of which are incorporated herein by reference. --

PATENT
Attorney Docket No. 110.01270101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): James B. McCarthy, et al.)
Serial No.: Unknown)
Filed: Concurrently Herewith)
For: METHODS OF USE OF β 1-INTEGRIN INHIBITORS)

SUBMISSION OF FORMAL DRAWINGS
UNDER 37 C.F.R. §1.85

BOX PCT

Assistant Commissioner for Patents
Washington D.C. 20231

Attached please find sixteen (16) sheets of formal drawings for the above-identified patent application. Please contact the undersigned attorney if you have any questions.

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Name: MARC IRELAND

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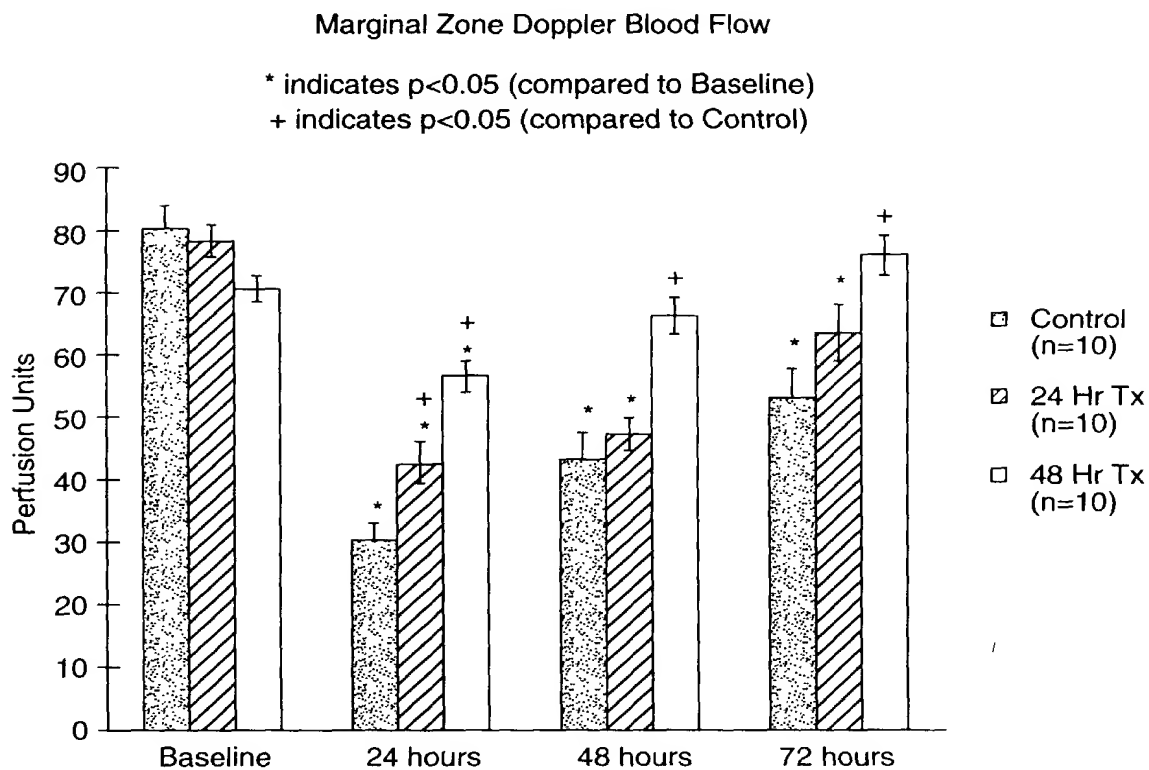


Fig. 1

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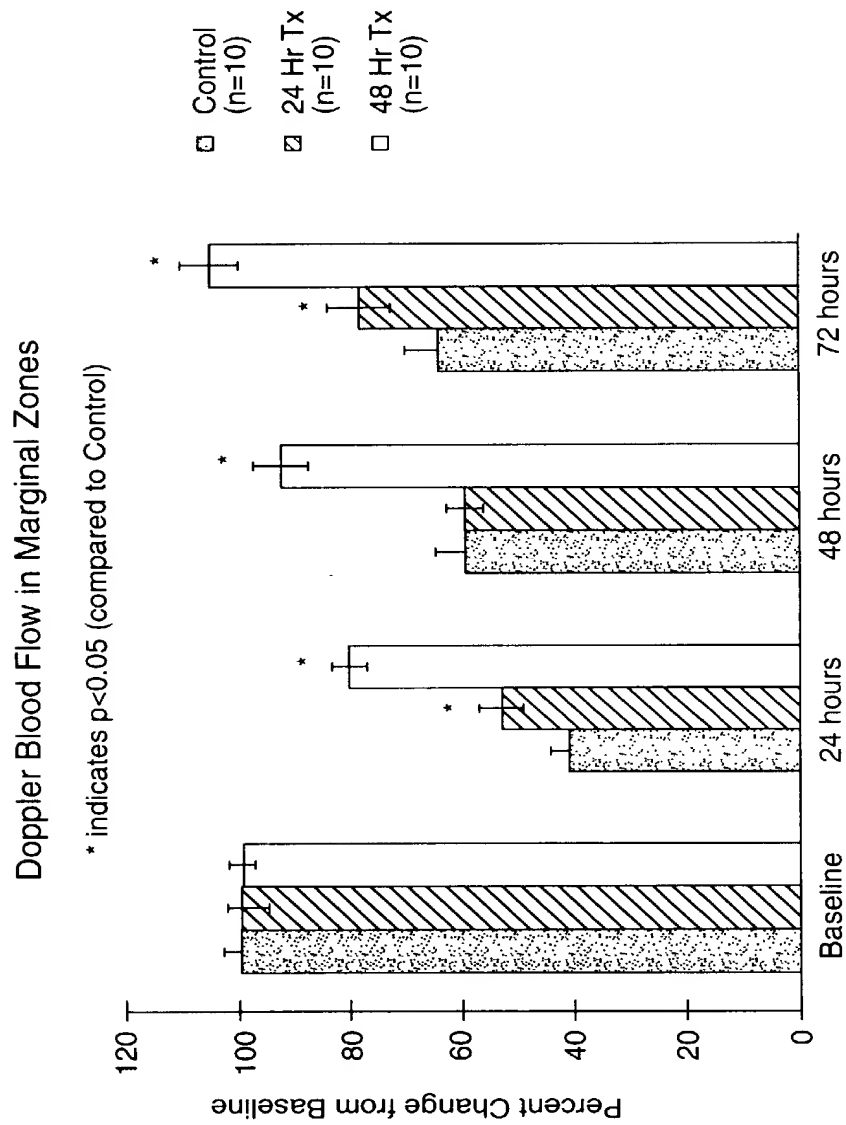


Fig. 2

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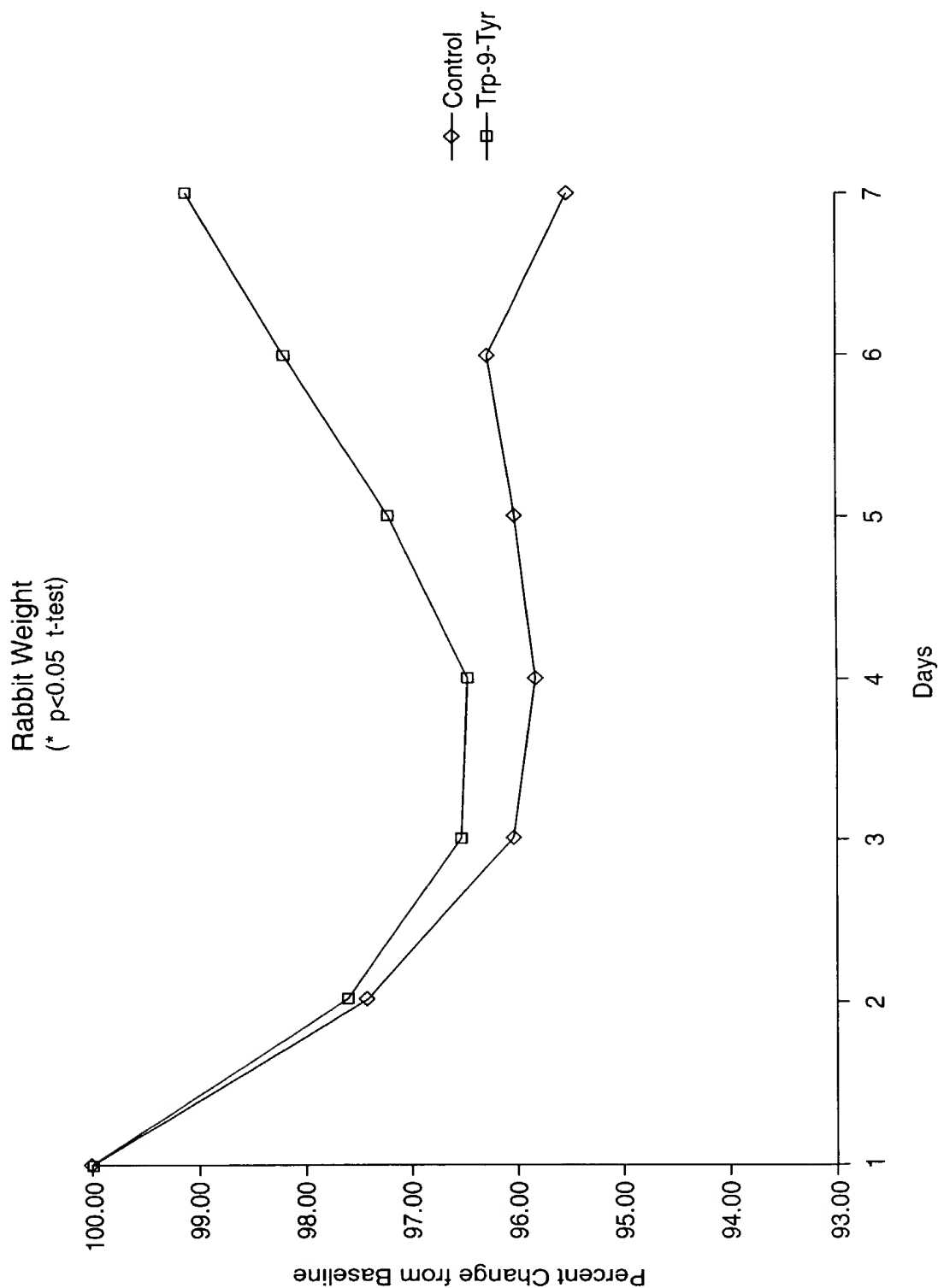


Fig. 3

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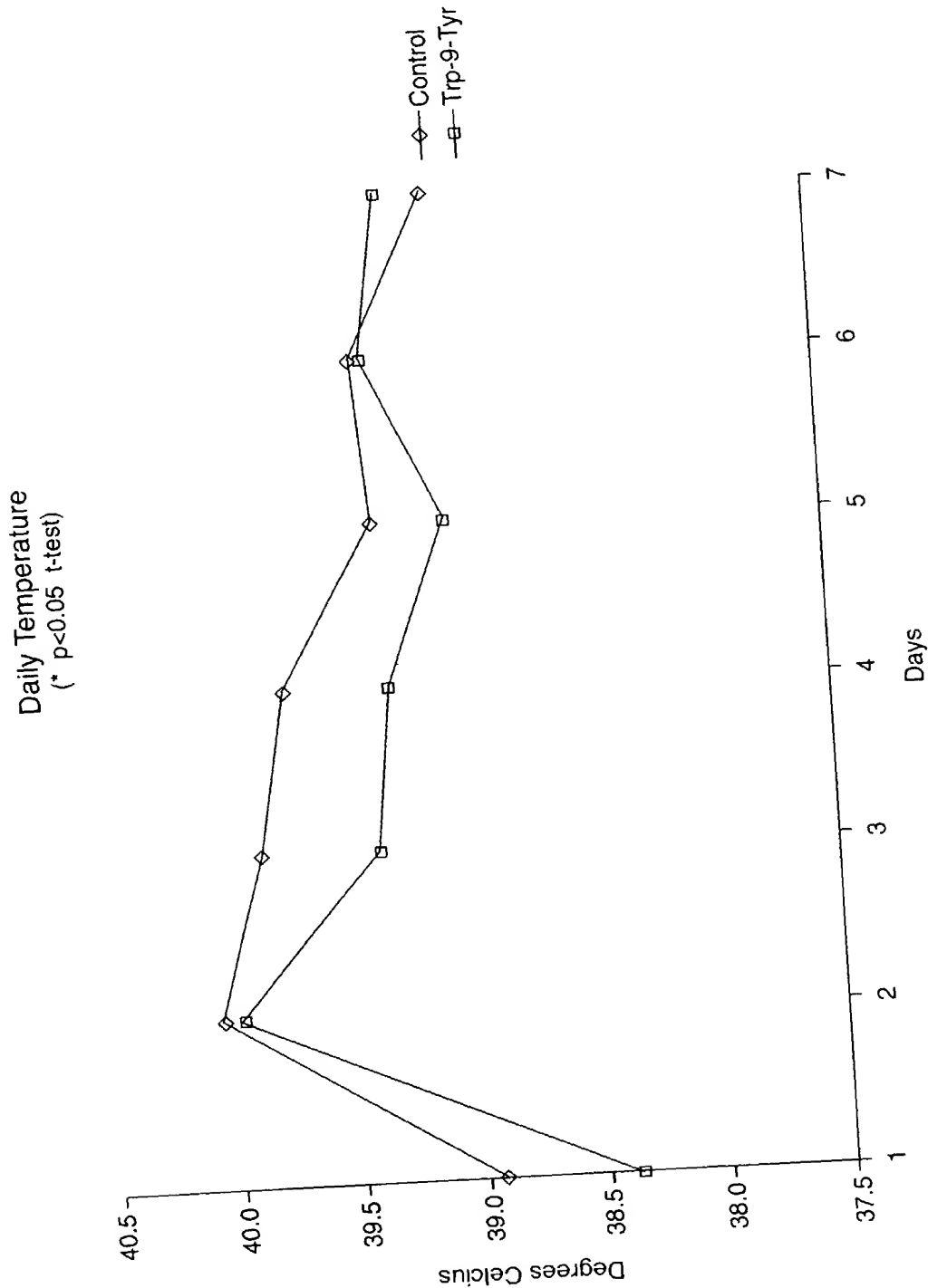


Fig. 4

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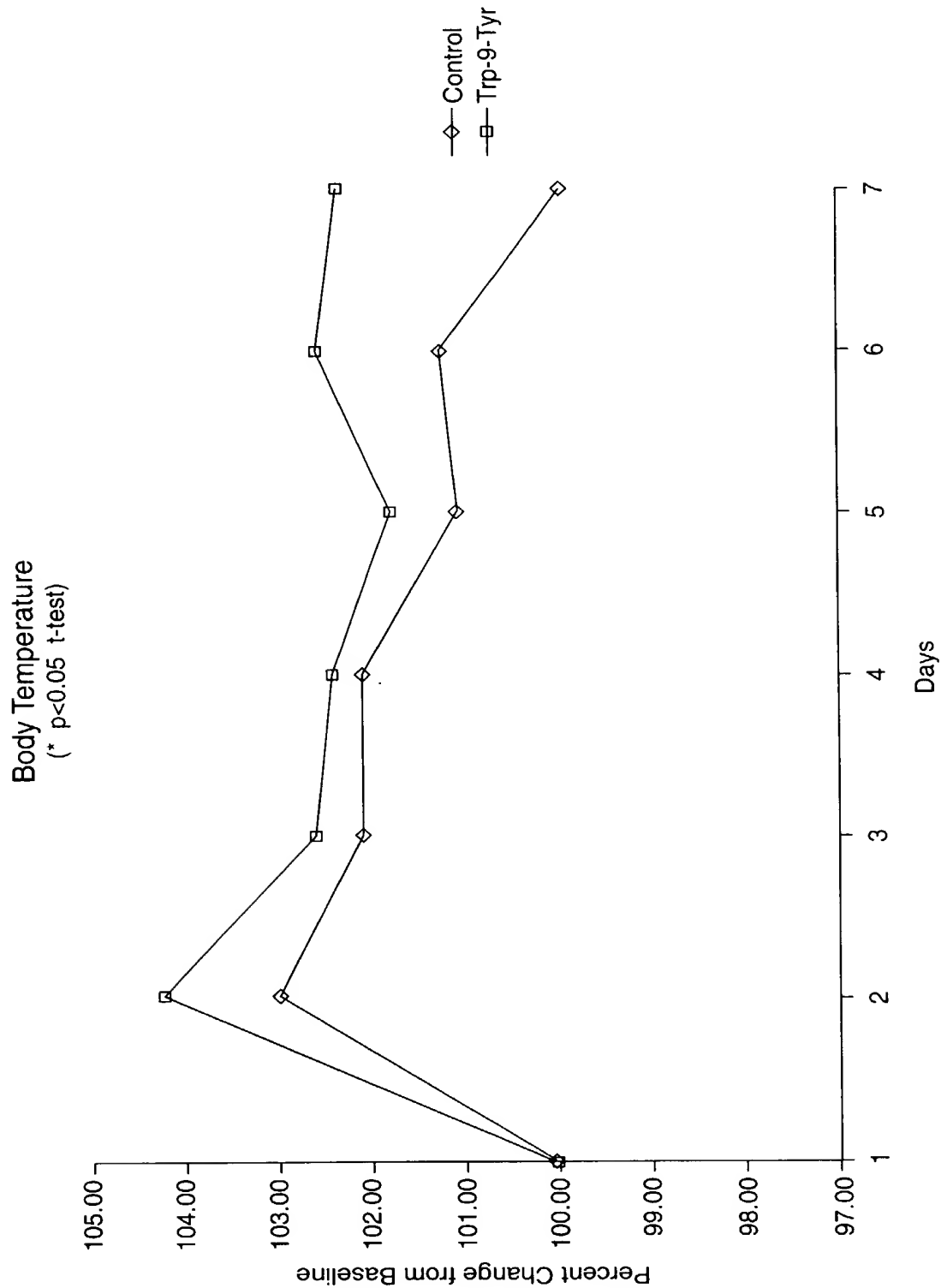


Fig. 5

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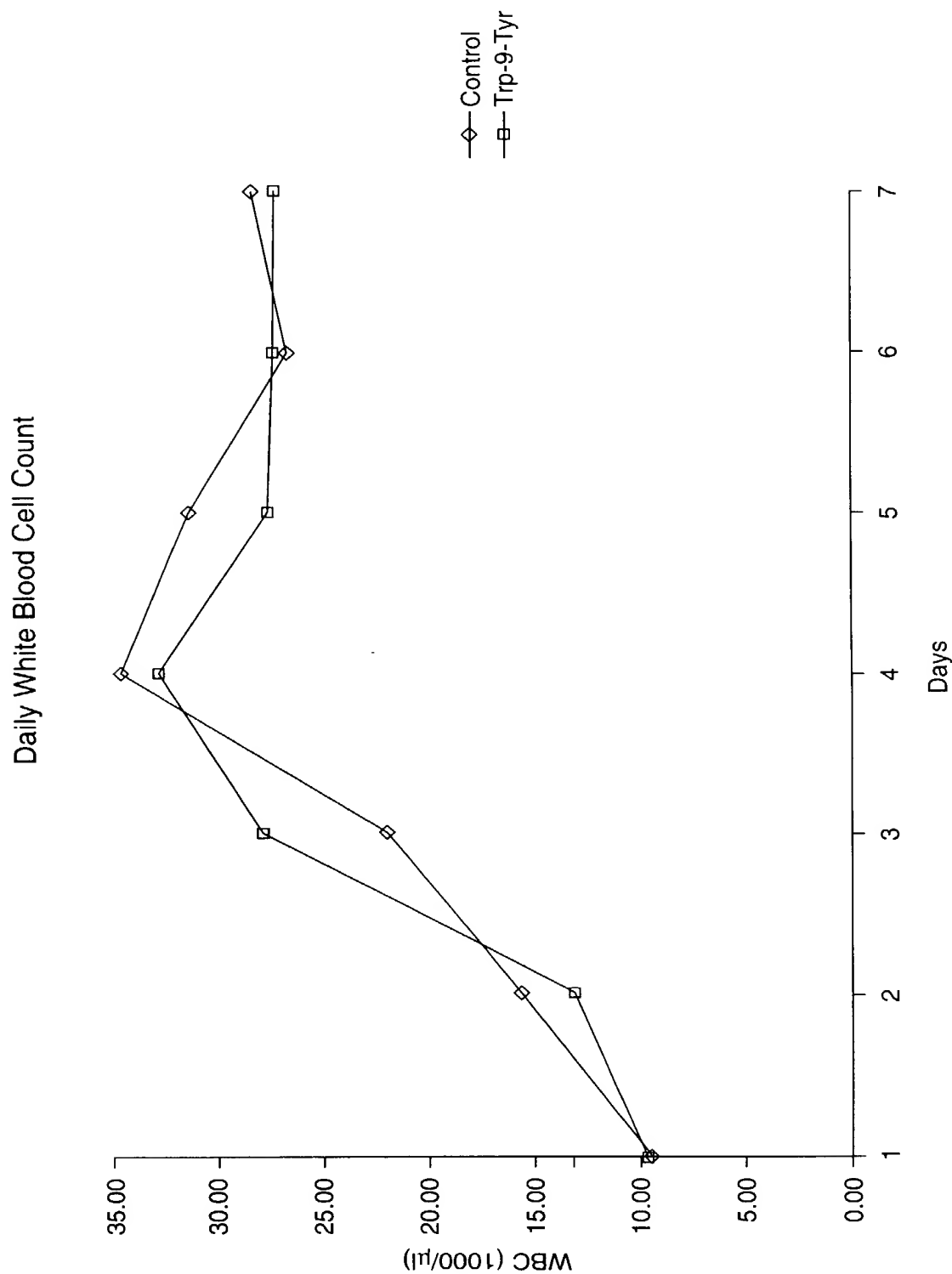


Fig. 6

Title: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

Applicant(s) James B. McCarthy, et al

Serial No. Not yet assigned Filed. Even date herewith

Docket 110 01270101

Express Mail No. EL 888271988 US

Sheet 7 of 16

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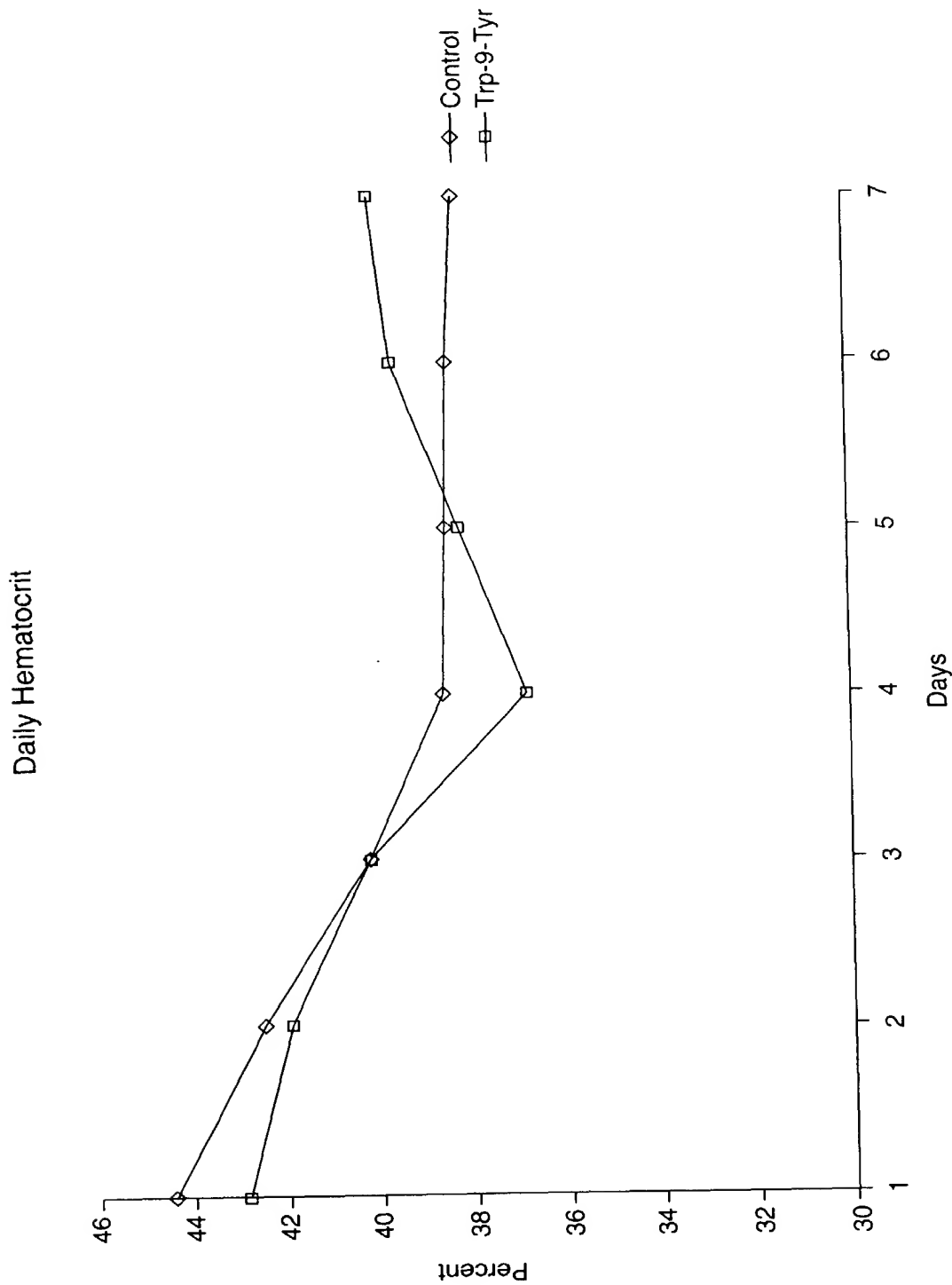


Fig. 7

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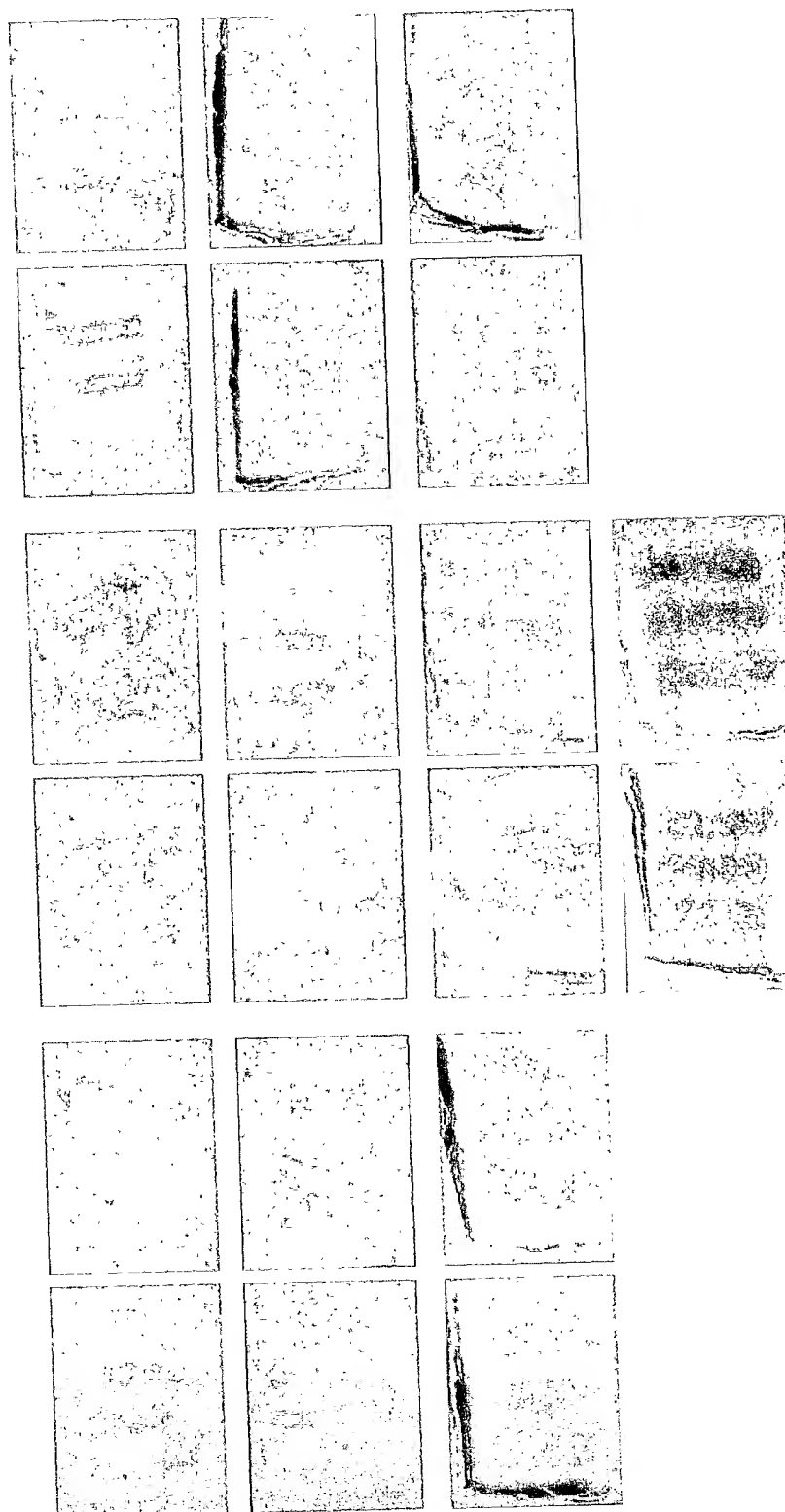


Fig. 8

Title METHODS OF USE OF $\beta 1$ -INTEGRIN INHIBITORS

Applicant(s): James B. McCarthy, et al

Serial No.: Not yet assigned

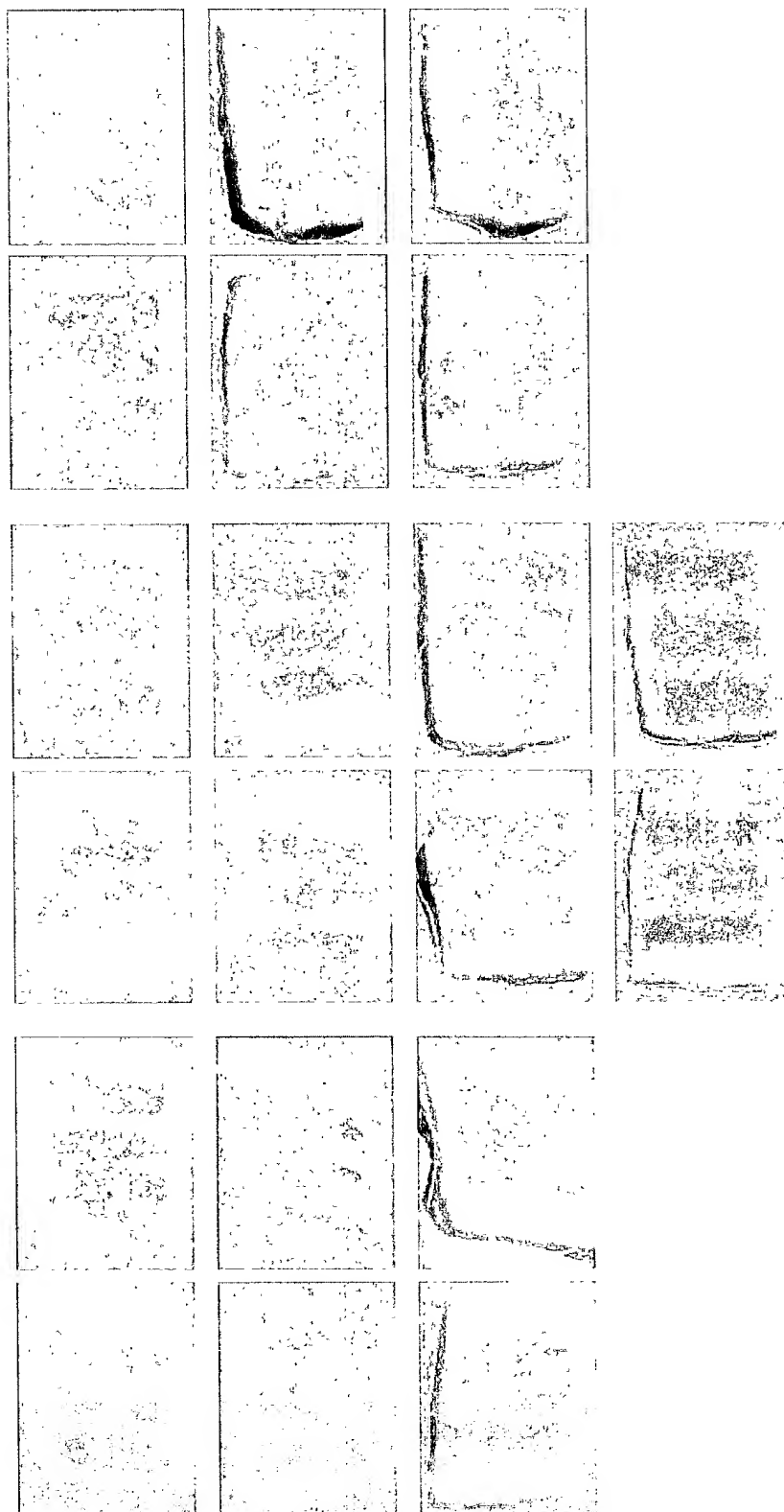
Filed: Even date herewith

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*Fig. 9*

09/937076

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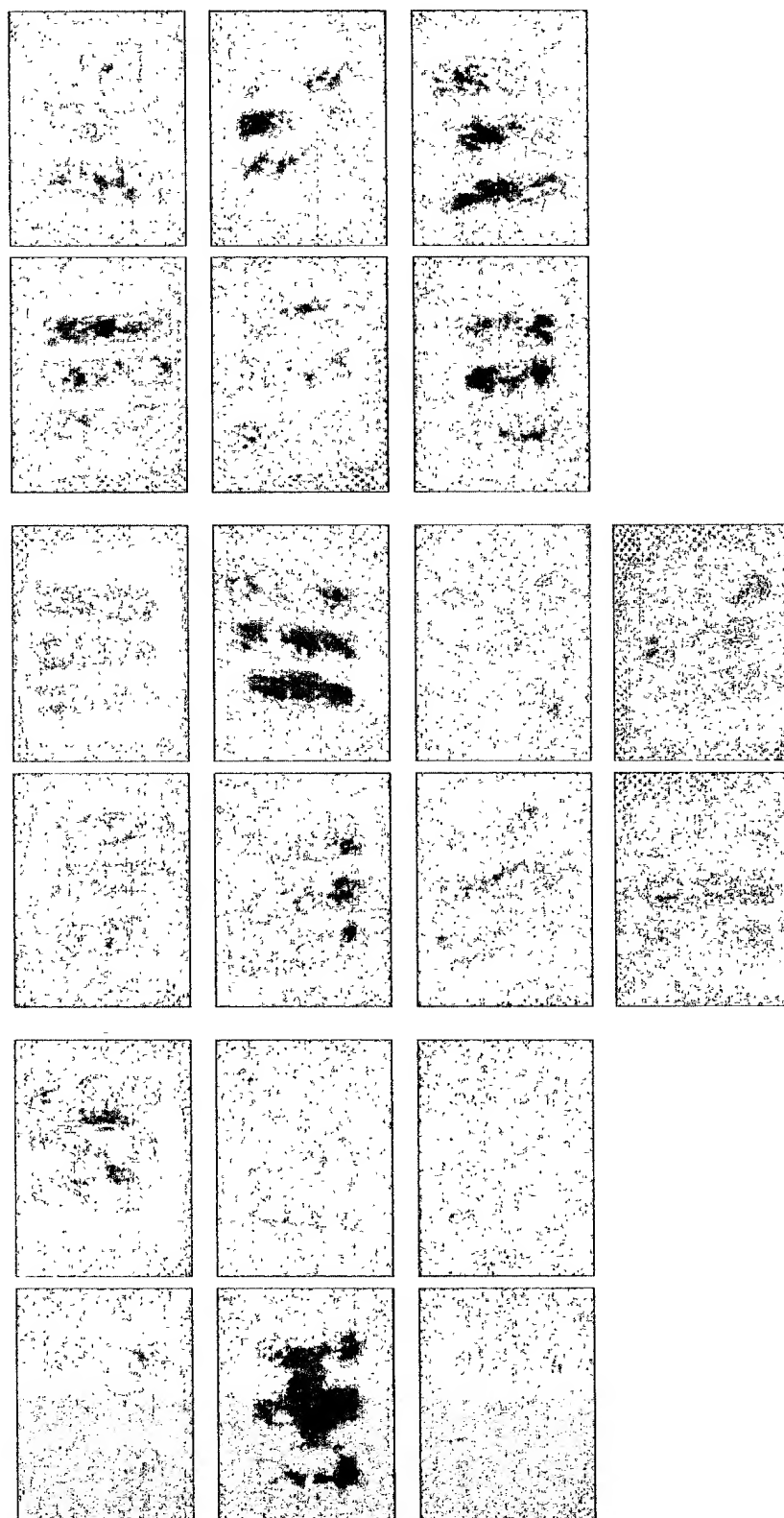


Fig. 10

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| Abscess Formation at Day 7 | | | | | | | | | |
|----------------------------|---|---|---|---|----|-----|------|--|--|
| Control | 1 | 7 | 8 | 9 | 10 | SUM | Mean | | |
| Rabbit | 2 | 2 | 2 | 2 | 2 | 10 | 2 | | |
| 10 ⁹ | 1 | 0 | 1 | 2 | 1 | 5 | 1 | | |
| 10 ⁸ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 10 ⁷ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 10 ⁶ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Trp-9-Tyr | | | | | | | | | |
| Rabbit | 2 | 3 | 4 | 5 | 6 | | | | |
| 10 ⁹ | 2 | 2 | 2 | 2 | 2 | 10 | 2 | | |
| 10 ⁸ | 0 | 1 | 2 | 0 | 1 | 4 | 0.8 | | |
| 10 ⁷ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 10 ⁶ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |

Fig. 11

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| Abscess Size at Day 7 | | (Area=square cm) | | | | | | | |
|-----------------------|-------|------------------|-------|-------|-------|-------|-------|--------|--------|
| Control | | 1 | 7 | 8 | 9 | 10 | Mean | sd | SEM |
| Rabbit | | 9.00 | 12.71 | 23.00 | 19.74 | 56.16 | 24.12 | 18.747 | 8.407 |
| 10 ⁹ | | 8.10 | 11.52 | 15.84 | 19.76 | 52.00 | 21.44 | 17.638 | 7.910 |
| 10 ⁸ | | 0.25 | 0.00 | 8.00 | 8.91 | 1.70 | 3.77 | 4.336 | 1.944 |
| 10 ⁸ | | 0.00 | 0.00 | 0.00 | 0.80 | 0.00 | 0.16 | 0.358 | 0.160 |
| Total | | | | | | | | | |
| mean 10 ⁹ | | 8.55 | 12.12 | 19.42 | 19.75 | 54.08 | 22.78 | 18.141 | 8.135 |
| mean 10 ⁸ | | 0.13 | 0.00 | 4.00 | 4.86 | 0.85 | 1.97 | 2.290 | 1.027 |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| Trp-9-Tyr | | | | | | | | | |
| Rabbit | 2 | 3 | 4 | 5 | 6 | Mean | sd | SEM | t-test |
| 10 ⁹ | 13.75 | 11.55 | 20.21 | 17.20 | 16.50 | 15.84 | 3.322 | 1.490 | 0.179 |
| 10 ⁹ | 12.90 | 16.00 | 18.80 | 12.32 | 20.00 | 16.00 | 3.428 | 1.537 | 0.241 |
| 10 ⁸ | 0.00 | 0.35 | 5.20 | 0.00 | 1.40 | 1.39 | 2.206 | 0.989 | 0.119 |
| 10 ⁸ | 0.00 | 0.00 | 2.52 | 0.00 | 0.00 | 0.50 | 1.127 | 0.505 | 0.288 |
| Total | | | | | | | | | |
| mean 10 ⁹ | 13.33 | 13.78 | 19.51 | 14.76 | 18.25 | 15.92 | 2.782 | 1.248 | 0.204 |
| mean 10 ⁸ | 0.00 | 0.18 | 3.86 | 0.00 | 0.70 | 0.95 | 1.653 | 0.741 | 0.174 |

Fig. 12

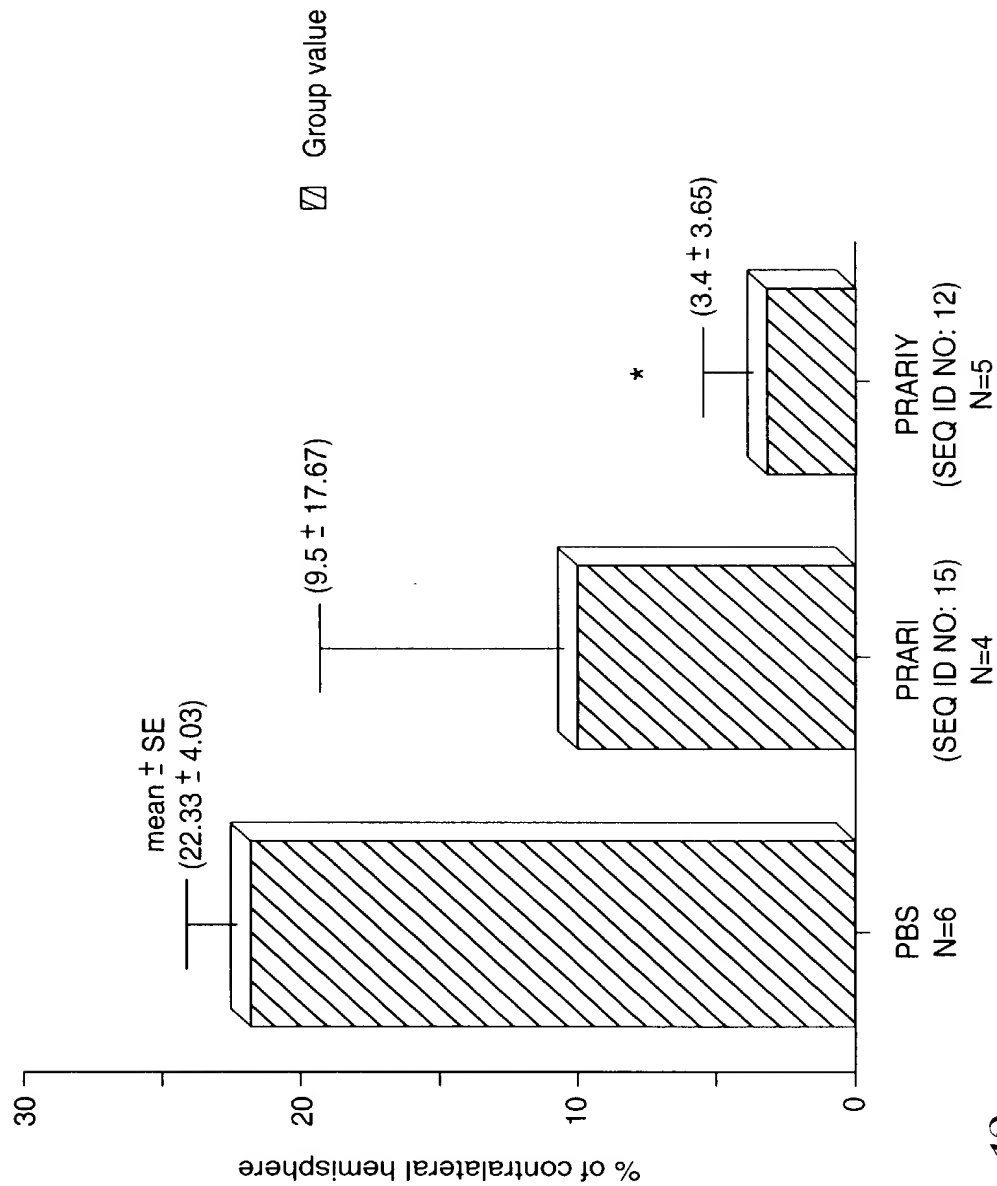


Fig. 13

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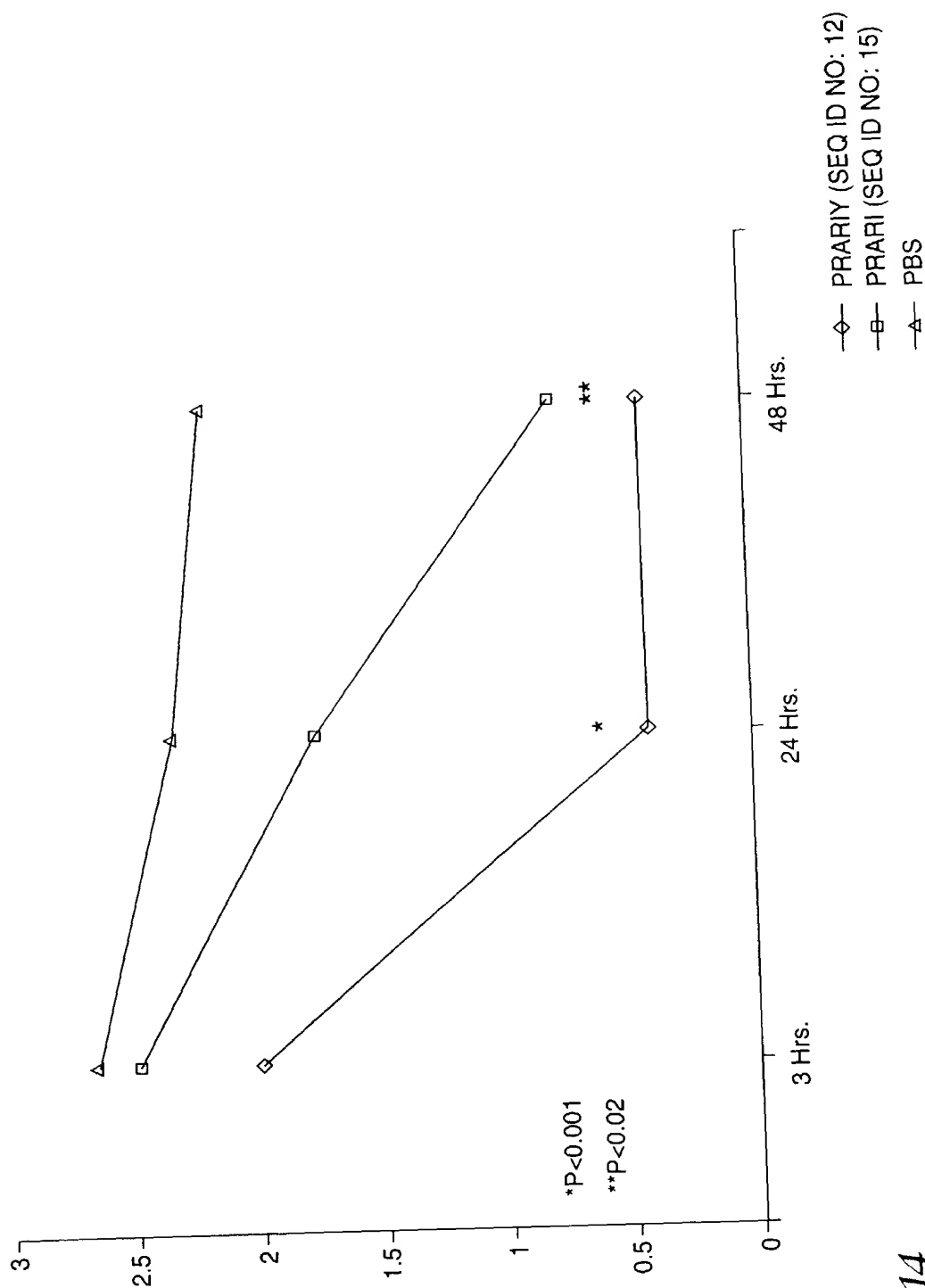


Fig. 14

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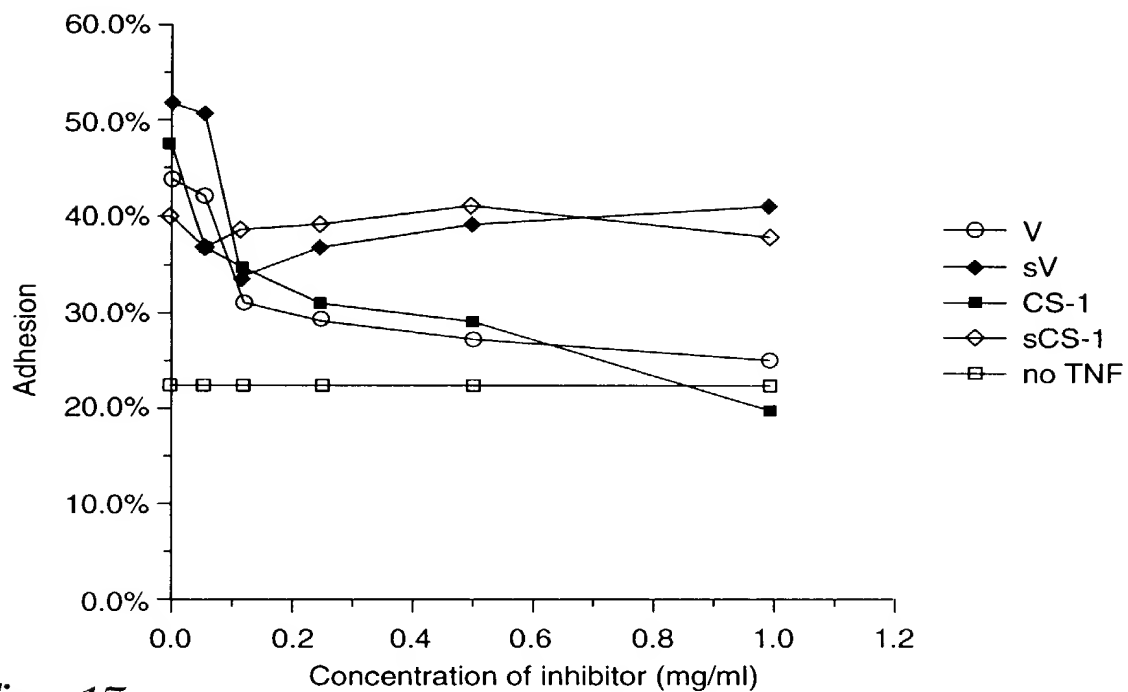


Fig. 15

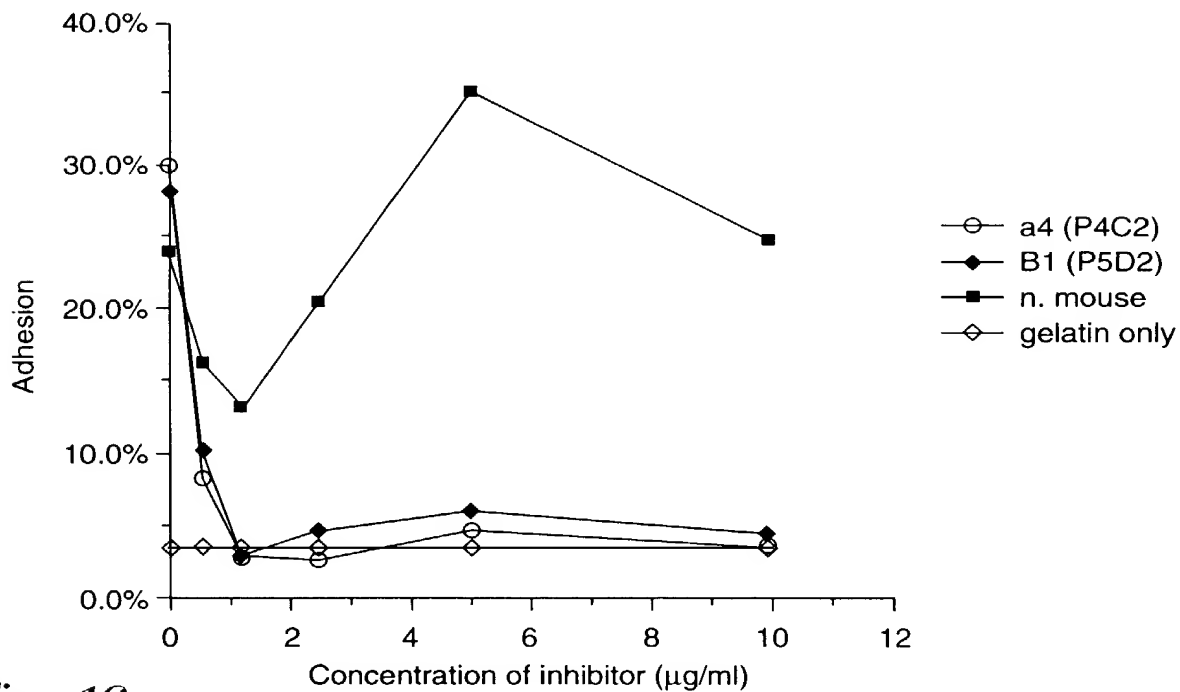


Fig. 16

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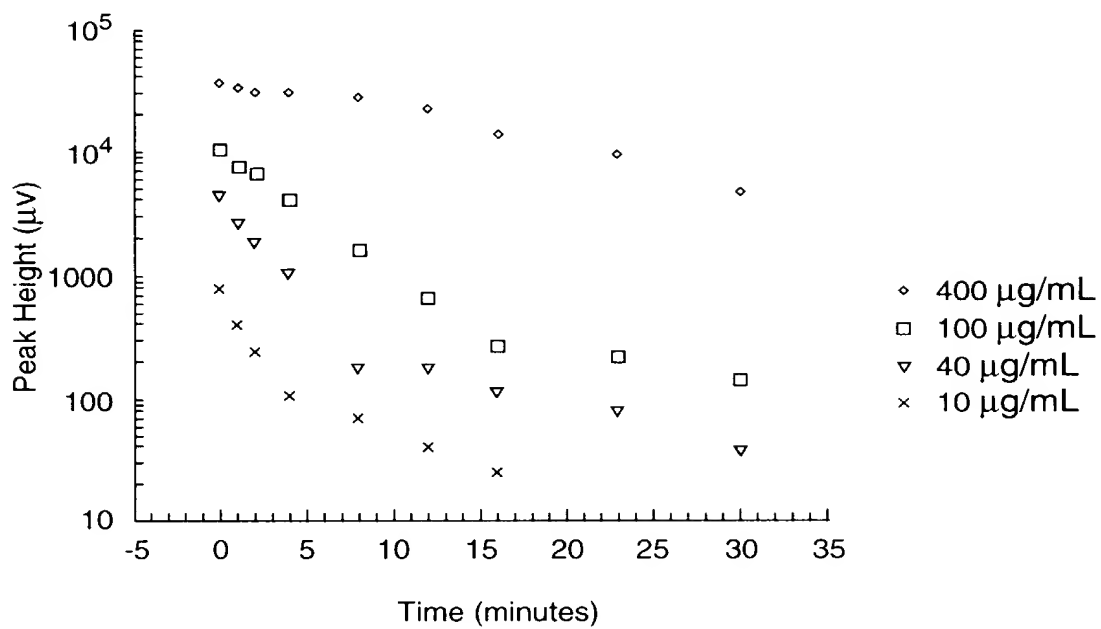


Fig. 17

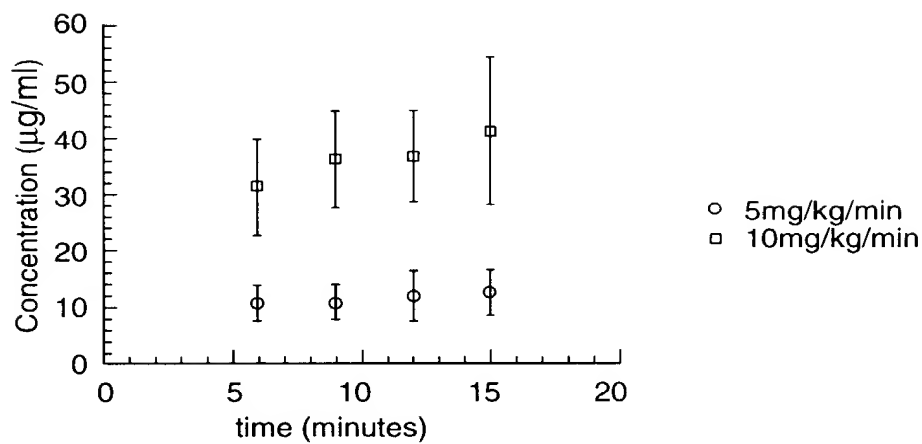


Fig. 18

METHODS OF USE OF β 1-INTEGRIN INHIBITORS

5

Statement of Related Applications

The present invention claims priority to U.S. Provisional Application Serial No. 60/125,634, filed March 22, 1999, and U.S. Provisional Application Serial No. 60/167,538, filed November 24, 1999, both of which are incorporated herein by
10 reference.

Background of the Invention

Integrins are a family of cell surface proteins that mediate the interactions of cells with their environment. They are heterodimeric proteins that include two
15 membrane glycoproteins, a larger α subunit and a smaller β subunit. The β_2 subunits are largely involved in cell-cell interactions, while β_1 subunits are associated with mediating cell adhesion to extracellular matrix constituents, including extracellular matrix (ECM) macromolecules reactive with β_1 subunit containing integrin molecules, such as fibronectin, laminin, tenascin, and Type IV
20 collagen. See, for example, International Publication No. WO 99/37669.

As cell surface proteins, integrins regulate a variety of cellular interactions including cellular adhesion to extracellular matrices, cell-cell association, and cellular motility/migration within tissues/organs. They also are considered
important mediators of a variety of pathological conditions, including acute
25 inflammation, cancer, and osteoporosis, and serve to localize and/or promote development/differentiation of cells within specific tissue and/or organ compartments, e.g., stem cell localization and differentiation.

Inflammation

30 Inflammation is required to orchestrate the recovery of tissue integrity, immune competence, and homeostasis following severe injury. Acute inflammation

represents the first step of this biological process; however, if the inflammatory response is excessive in initial magnitude or duration, this otherwise beneficial process can contribute to the further deterioration rather than the restoration of tissue/organ homeostasis.

5 Acute inflammation induced by CNS ischemic injury and thermal/cold injury represent two clinical conditions wherein this two-edged sword of inflammation operates. In each of these situations, similar events occur subsequent to the reperfusion of the injured tissue(s). These events determine whether the injury extends outward from the initial focus of the injury to include additional tissue, or
10 recover, thus containing the injury to the initial site and limiting any additional loss of functional tissue. In addition to burns and stroke, a wide variety of clinical situations share this inflammation-mediated progression of injury size and scope, including myocardial infarction, angioplasty, surgical incisions, injury-mediated trauma and transplant reperfusion.

15 Much of the acute inflammatory response within tissues is mediated by leukocytes. That is, leukocytes have been implicated in the pathogenesis and progression of microvascular injury and extravascular tissue damage. To reach the site of injury, leukocytes must adhere to the vessel/capillary wall and then migrate to the injured tissue. Thus, leukocyte-mediated injury is dependent in part on
20 polymorphonuclear neutrophil adherence to endothelial cell surface and leukocyte aggregation in extracellular tissue.

 Leukocyte adherence to endothelial cells and to extracellular matrix components is mediated by multiple adhesion receptor systems. In the first stages of inflammation, leukocyte rolling into inflammatory sites is mediated by the
25 selectin family of adhesion receptors. Additional cellular recognition receptors, the integrins, then mediate leukocyte binding to the endothelium. Once leukocytes migrate across the endothelial cell membrane their adherence to the extracellular matrix is directed by many factors, including integrins.

 There is increasing evidence that integrins represent one important set of
30 mediators of the pathological events associated with acute inflammation. In

particular, β 1-integrins have recently been determined to play a critical role in the adhesion and migration of leukocytes (PMNs) into tissues at the site of injury, as the expression of β 1-integrins is elevated 25-fold on extravasated PMNs over the level of β 1-integrins expressed on circulating PMNs. Similar elevation of β 1-integrin expression is observed in other biological and/or pathological conditions.

Cancer

β 1-integrins also play important roles in cancer cell differentiation, tumor metastasis, angiogenesis, and regulation of tumor cell apoptosis-modulators of cancerous tissue growth and spread. As increased expression of β 1-integrins has been demonstrated to correlate with prognosis and metastatic potential (i.e., higher levels of β 1-integrins correlate with higher metastatic potential), β 1-integrins appear to coordinately regulate critical parameters within cancerous tissue. The levels of β 1-integrin are often increased on cancerous cells/tissues, thus altering the interactions of cancer cells with their surroundings and inducing changes in the cancer cell's phenotype.

Osteoporosis

Integrins mediate the interactions of osteoclasts with their environment and thus the actions of osteoclasts. Of the several integrins expressed on the surfaces of osteoclasts, β 1-integrins are one of those expressed at the highest levels. Recent studies have determined β 1-integrin activity to correlate with the adhesive and bone-resorptive capacity of osteoclasts.

Stem Cell Localization

Bone marrow transplant (either allogeneic or autologous) represents an increasingly utilized approach to managing a variety of hematopoietic and cancerous disease states. Current methodologies require the direct harvesting of donor bone marrow, a painful and invasive medical procedure. This approach limits the availability of donor stem cell materials. Enhanced methods of obtaining

hematopoietic stem cells, for example, by cellular phoresis, would increase the utilization of bone marrow transplant as a strategy for the treatment of cancer, AIDS, and genetic disease (gene therapy).

5 There are numerous agents that have been described that modulate integrin activity. Only two classes of agents, however, have been described that interact both selectively in the extracellular space with the β subunit of β 1-integrins and possess the capacity to inhibit the activity of one or more integrin heterodimer complexes. These two classes include antibodies and the peptides disclosed in
10 International Publication No. WO 99/37669, the latter of which are the only ones known to interact preferentially for activated β 1-integrins. Inhibitory antibodies are known to possess the capability of binding and blocking the activity of integrin β 1 subunits; however, these tend to be large and not very accessible to pathological sites.

15 Thus, there is a need for additional agents that modulate (e.g., inhibit) integrin activity, particularly those that have better access to pathological sites than antibodies. For example, there is still a need in the art for other agents that can inhibit inflammatory leukocyte mediated destruction of tissues, especially during ischemia reperfusion injury resulting from CNS ischemic injury (e.g., stroke),
20 myocardial infarction, angioplasty, surgical incisions, injury-related trauma, and transplant reperfusion. There is still a need for agents that can inhibit inflammatory leukocyte mediated destruction of tissues subsequent to injuries induced by exposure to heat, cold, light, electricity, chemicals, or other agents that cause tissue injury and destruction.

25 In addition, there is still a need for agents that can: inhibit angiogenesis; inhibit cancer cell metastasis, motility, and/or migration within tissues; and restore/potentiate the induction of programmed cell death in cancerous tissues. Also, there is still a need for agents that can inhibit osteoclast adhesion and bone resorption. Furthermore, there is still a need for agents that can promote the
30 peripheralization of hematopoietic stem cells for the purposes of harvesting said

stem cells and using them for various transplant procedures and methodologies including autologous stem cell transplantation to restore hematopoietic potential subsequent to cyto-reductive therapies (cancer chemo- and radio- therapeutics and the like), or for the purposed of generating specific immune cells for use in the
5 production of cell based immunogens for cancer, viruses, pathogens and the like.

Summary of the Invention

The present invention provides methods that involve the use of $\beta 1$ -integrin inhibitors. In one embodiment, a method of inhibiting inflammatory leukocyte
10 mediated destruction of tissue in a patient is provided. The method includes administering to the patient a composition comprising a $\beta 1$ -integrin inhibitor. As used herein, a $\beta 1$ -integrin inhibitor is an agent that inhibits $\beta 1$ subunit containing integrin irrespective of the associated α subunit.

In this method, the inflammatory leukocyte mediated destruction of tissue
15 can occur as a result of CNS ischemic injury, myocardial infarction, angioplasty, surgical incisions, injury-related trauma, transplant reperfusion, or a combination thereof. Alternatively, the inflammatory leukocyte mediated destruction of tissue can occur as a result of exposure to heat, cold, light, electricity, chemicals, or a combination thereof. The injury that results from any of these conditions is referred
20 to herein as a "burn-type" injury.

In a preferred embodiment of the present invention, there is provided a method of treating a stroke patient. The method includes administering to the
patient a composition that includes a $\beta 1$ -integrin inhibitor in an amount effective to
reduce infarct size, reduce neurological deficit, or both. Preferably, such
25 administration occurs within about 3 hours after the stroke occurs.

In another preferred embodiment, there is provided a method of treating a patient having a burn-type injury. The method includes administering a
composition that includes a $\beta 1$ -integrin inhibitor in an amount effective and over a
period of time effective to reduce leukocyte-mediated tissue destruction. The period
30 of time is preferably at least 1 hour. Also, preferably the administration is topical.

29-03-2001

ARTICLE 34

In certain embodiments, the composition is administered periodically over a predetermined period of time.

In yet another embodiment, there is provided a method of treating a burn patient that involves maintaining a composition that includes an effective amount of a $\beta 1$ -integrin inhibitor on a burn-type injury for a period of time effective to reduce leukocyte-mediated tissue destruction and achieve a desired degree of healing.

The present invention also provides a method of treating a cancer patient. The method involves administering to the patient a composition that includes a $\beta 1$ -integrin inhibitor in an amount effective to inhibit one or more of angiogenesis, cancer cell metastasis, cancer cell motility, or cancer cell migration. Alternatively, the method involves administering a $\beta 1$ -integrin inhibitor in an amount effective to induce programmed cell death in cancerous tissue or restore normal cellular phenotype to cancerous tissue.

A method of treating a patient for osteoporosis is also provided. The method involves administering to the patient a composition that includes a $\beta 1$ -integrin inhibitor in an amount effective to inhibit osteoclast adhesion and bone resorption.

A method of peripheralizing stem cells is also provided. This method involves administering to a patient a composition comprising a $\beta 1$ -integrin inhibitor.

Yet another embodiment of the present invention is a composition that includes a $\beta 1$ -integrin inhibitor and a pharmaceutically acceptable carrier. As used herein, "a" means one or more, such that combinations of inhibitors can be used in the compositions and methods of the invention.

Preferably, the $\beta 1$ -integrin inhibitor is a peptide that has a C-terminal LipAr motif. More preferably, the $\beta 1$ -integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAARIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYTY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWIY

(SEQ ID NO:11), PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.

Brief Description of the Figures

5 The invention can be better understood with reference to the following detailed description together with the appended illustrative drawings in which like elements are numbered the same:

Figure 1 is a bar chart of the Doppler blood flow in marginal zones of burn study. This shows blood flow in burn zones at baseline, 24, 48, and 72 hours.

10 Control – 80.56 ± 3.58 , 29.97 ± 2.94 , 42.67 ± 4.38 , 52.17 ± 5.06 ; 24 hour treatment – 78.33 ± 2.64 , 42.25 ± 3.40 , 46.63 ± 2.69 , 62.62 ± 4.69 ; 48 hour treatment – 70.69 ± 2.07 , 56.23 ± 2.55 , 65.70 ± 2.95 , 75.19 ± 3.30 . Laser Doppler perfusion measurement in zones of stasis presented as mean \pm SEM for controls (solid bar), 24-hour treatment group (broken bar) and 48-hour treatment group (white bar) (*p < 0.05 versus Baseline by t-test; +p < 0.05 versus Control by t-test). Control animals

15 had significant decreases in perfusion in the zones of stasis at all post-burn time points. Animals in the 24-hour treatment group had higher perfusion than controls at 24-hour post burn. Animals in the 48-hour treatment group had higher blood flow in the zones of stasis at 24, 48, and 72 hours versus controls.

20 Figure 2 is a bar chart of marginal zone Doppler blood flow.

Figure 3 is graph of data showing rabbit weight changes for seven days.

Figure 4 is graph of data showing daily temperature changes for seven days.

Figure 5 is graph of data showing body temperatures for seven days.

25 Figure 6 is graph of data showing daily white blood cell count for seven days.

Figure 7 is graph of data showing daily hematocrit for seven days.

Figure 8 is a photograph of the zones of stasis in a group of control animals.

Figure 9 is a photograph of the zones of stasis in a group of animals treated for 24 hours with Trip-9-Tyr.

30 Figure 10 is a photograph of the zones of stasis in a group of animals treated

for 48 hours with Trp-9-Tyr.

Figure 11 is table of data showing abscess formation at day 7 in peptide side-effect study.

Figure 12 is table of data showing abscess size at day 7 in peptide side-effect study.

Figure 13 is a bar chart of infarction size of MCAO (stroke) study.

Figure 14 is a bar chart of neurological index results of MCAO (stroke) study.

Figure 15 is a graph of the inhibition of Ramos Adhesion to tumor necrosis factor alpha-stimulated human vascular endothelial cells; V = WQPPRARIY (SEQ ID NO:1); sV = scrambled (inactive) version of V which has an amino acid sequence of RPQIPWARY (SEQ ID NO:16); sCS-1 = scrambled version of CS-1; TNF = tumor necrosis factor alpha.

Figure 16 is graph of the inhibition of Ramos Adhesion to tumor necrosis factor alpha-stimulated human vascular endothelial cells; a4 = inhibitory antibody against the alpha-4 integrin molecule; B1 = β 1 inhibitory antibody; n. mouse = normal mouse serum.

Figure 17 is a graph of the time course of WQPPRARIY (SEQ ID NO:1) in human plasma at 37°C with varying initial concentrations.

Figure 18 is a graph of average (SD) concentrations of WQPPRARIY (SEQ ID NO:1) in rat blood versus time during two-phase crossover IV infusion study. The rats were given a low dose treatment (o) first at 5 mg/kg/min for 15 minutes, and immediately after this, the animals received a high dose infusion (\square) at 10 mg/kg/min for 15 minutes.

Detailed Description of Preferred Embodiments of the Invention

The present invention provides methods that use agents capable of one or more of the following: inhibiting leukocyte mediated destruction of tissues, especially after injuries to tissue due to heat or cold (i.e., burns or frostbite), light, electricity, chemicals, or other agents that cause tissue injury and destruction; inhibiting leukocyte mediated destruction of tissues after myocardial infarction, CNS ischemic injury (e.g., stroke), angioplasty, surgical incisions, injury-mediated

trauma, and transplant reperfusion; inhibiting angiogenesis; inhibiting cancer cell metastasis, motility, and/or migration within tissues; restoring and/or potentiating the induction of programmed cell death in cancerous tissues or restoring normal cellular phenotype to cancerous tissue; inhibiting osteoclast adhesion and bone
5 resorption; and/or inhibiting stem cell adhesion to bone marrow.

The agents useful in the methods of the present invention are those that interact selectively with activated $\beta 1$ -integrin molecules (i.e., in the conformational state that permits/promotes interaction with ligand, which in this case are components of the extracellular matrix, such as fibronectin, laminin, collagen and
10 the like, and/or cell surface molecules) relative to resting (i.e., unactivated) $\beta 1$ -integrin molecules. They are preferably smaller than antibodies to $\beta 1$ -integrins. Generally, monomeric IgG molecules (antibodies) are approximately 150 kDa while the active agents used herein are no greater than about 15 kDa, preferably no greater than about 5 kDa, and more preferably no greater than about 1000 daltons (1 kDa).

15 Furthermore, agents useful in the methods of the present invention do not induce an immuno-suppressed state. Significantly, selectivity for activated $\beta 1$ -integrin molecules may allow for systemic administration and the use of higher doses. Also, such agents are advantageous because they are believed to induce fewer side effects. Examples of such agents are described in International
20 Publication No. WO 99/37669. These agents, as well as others (which may or may not be peptides) that can be developed based on structure-activity relationships defined by these agents, are useful in the methods of the present invention.

The methods of the present invention include treatments for inflammation (e.g., such as that resulting from burns), cancer, and osteoporosis, as well as
25 methods of harvesting hematopoietic stem cells.

Inflammation

Integrins represent an important set of mediators of acute inflammation, with $\beta 1$ -integrins playing an important role in the adhesion and migration of leukocytes
30 into tissues at the site of injury. Moreover, the expression of $\beta 1$ -integrins is elevated

25-fold on extravasated leukocytes over the level of $\beta 1$ -integrins expressed on circulating leukocytes. As such, inhibition of $\beta 1$ -integrin activity would likely provide an approach to mitigating the adverse effects of the acute inflammatory response, as it would block the migration and/or inflammatory responses elicited by leukocytes within tissues. Moreover, as integrins possess limited biological redundancy, are late effectors of critical biological processes, and can be blocked with limited side effects agents which block integrin activity are likely to possess limited side effects. Thus, agents that modulate $\beta 1$ -integrin activity are believed to provide superior therapeutic regulation of acute inflammation processes.

The agents of the present invention are believed to provide superior access to sites of inflammation. They also provide the structural basis/rationale for the development of small molecules (peptides, peptiomimetics, and/or small organic molecules) possessing enhanced anti- $\beta 1$ -integrin inhibitory activity.

A wide variety of clinical situations that share an inflammation-mediated progression of injury size and scope include surgical incisions, injury-mediated trauma, myocardial infarction, angioplasty, CNS ischemic injury (e.g., stroke), transplant reperfusion, heat, cold, light, electricity, chemicals, etc., can also be positively affected by the agents of the present invention. Other clinical situations can include injuries initiated by heat or cold (i.e., burns or frostbite), light, electricity, chemicals, or other agents that cause tissue injury and destruction. Thus, the present invention provides a method of inhibiting inflammatory leukocyte mediated destruction of tissue in a patient by administering to the patient an effective amount of a $\beta 1$ -integrin inhibitor.

In a preferred embodiment, the present invention provides a method that utilizes one or more agents capable of ameliorating leukocyte-mediated tissue destruction without suppressing leukocyte activity against foreign agents such as bacterial or viral agents. Such method results from the discovery that inhibition of WBC adherence with Trp-9-Tyr reduces the microvascular damage and progression of tissue necrosis following thermal injury. The synthetic fibronectin peptide (Trp-9-Tyr) has the amino acid sequence WQPPRARIY (SEQ ID NO:1).

Specifically, the present invention provides a method of treating a patient having a burn-type injury by administering a $\beta 1$ -integrin inhibitor in an amount effective and over a period of time effective to reduce leukocyte-mediated tissue destruction. The order of preference for the period of time is at least 1 hour, at least
5 4 hours, at least 8 hours, at least 12 hours, at least 24 hour, at least 36 hours, and at least 48 hours. Preferably, the mode of administration is topical and the administration is periodic.

Cancer

10 Inhibition of $\beta 1$ -integrin activity has been demonstrated to induce phenotypic restoration in cancer cells *in vitro*. Furthermore, inactivating antibodies against $\beta 1$ -integrins have been demonstrated to block tumor cell mediated angiogenesis, a process involved in tumor growth and expansion. Also, inactivating antibodies against $\beta 1$ -integrins block the interaction of $\beta 1$ -integrins with the
15 extracellular matrix, which is rich in $\beta 1$ -integrin binding molecules, such as fibronectin, laminin, collagen, and tenascin that surround cancers, with $\beta 1$ -integrins, thereby restoring the induction of programmed cell death (apoptosis) in the cancerous cells and blocking cellular migration within tissues.

Thus, agents that modulate $\beta 1$ -integrin activity can serve as powerful
20 regulators of cancer cell biology. Thus, small molecule inhibitors (peptides, peptiomimetics, and/or small molecules) of $\beta 1$ -integrins, such as the agents of the present invention, are believed to provide superior access to tumor sites and permit the therapeutic regulation of cancer cell phenotype. They also provide the structural basis/rationale for the development of small molecules (peptides, peptiomimetics,
25 and/or small organic molecules) possessing enhanced anti- $\beta 1$ -integrin inhibitory activity. Induction/restoration of a normal cellular phenotype in tumor cells would provide for the development of superior cancer therapeutic regimens.

Cancers that can be treated using the methods of the present invention include, but are not limited to, breast cancer, melanoma, mesothelioma, and
30 myeloma. Such treatment methods can involve, for example, the inhibition of

tumor cell metastasis, the inhibition of tumor cell motility, the inhibition of tumor cell migration, the enhancement of tumor cell apoptosis, the induction of targeted paralysis and reversion of cancerous cell phenotype, and/or the inhibition of tumor mediated angiogenesis.

5 Thus, the present invention provides a method of treating a cancer patient by administering a β 1-integrin inhibitor in an amount effective to inhibit one or more of angiogenesis, cancer cell metastasis, cancer cell motility, or cancer cell migration.

10 Osteoporosis

 Improved methods of modulating osteoclast function are necessary to provide enhanced treatment methodologies for osteoporosis and other bone-resorptive disease processes. Agents that modulate β 1-integrin activity, such as those of the present invention, are believed to act as powerful regulators of
15 osteoclast function, thus facilitating the therapeutic regulation of bone-resorptive disease processes. Such agents are believed to provide superior access to the sites where bone turnover is occurring. They also provide structural basis/rationale for the development of small molecules (peptides, peptiomimetics, and/or small organic molecules) possessing enhanced anti- β 1-integrin inhibitory activity. Thus, the
20 present invention provides a method of treating a patient for osteoporosis by administering a β 1-integrin inhibitor in an amount effective to inhibit osteoclast adhesion and bone resorption.

Stem Cell Localization

25 Hematopoietic stem cells (CD34 cells) express high levels of β 1-integrins. These molecules mediate their adherence to bone marrow cavity (stroma). Agents that that modulate β 1-integrin activity (e.g., by blocking this interaction), such as the agents of the present invention, are believed to induce the peripheralization (movement into the circulation) of hematopoietic stem cells. This allows stem cells
30 to be harvested using standard cellular phoresis methodologies, for example, thus

providing a readily available supply of donor hematopoietic stem cells. Thus, such agents are believed to permit the development of enhanced hematopoietic stem cell harvesting techniques. It is further believed that this would increase the availability of donor materials, thus increasing the number and variety of procedures for which bone marrow transplantation could be employed.

Thus, methods of the present invention include the use of agents that enhance the peripheralization of stem cells through their interaction with activated forms of $\beta 1$ -integrins on stem cells. Such methods can be used in conjunction with known methods for harvesting cells from the peripheral circulation system.

Optionally, cytokines, such as G-CSF, GM-CSF and/or Flt 3 ligand, can be used that enhance stem cell peripheralization. Stem cells obtained according to the present invention can be used to treat a variety of conditions, including, but not limited to, cancer, AIDS, and genetic disorders through gene therapy.

Inhibitory Agents and Compositions

Suitable agents for use in the methods of the present invention include peptides with a C-terminal amino acid residue having a side chain that includes an aromatic group (“-Ar-”) and an amino acid residue with a lipophilic alkyl side chain group (“-Lip-”) as the penultimate C-terminal residue, as exemplified in International Publication WO 99/37669. This C-terminal dipeptide sequence is referred to herein as a “LipAr motif.” These peptides with a C-terminal LipAr motif and are typically capable of inhibiting $\beta 1$ -integrin subunit dependent cell adhesion and, in particular, of inhibiting $\alpha 4\beta 1$ -integrin dependent cell adhesion, and typically $\alpha 2\beta 1$, $\alpha 3\beta 1$, and/or $\alpha 5\beta 1$ integrin dependent cell adhesion.

Examples of suitable amino acid residues having an aromatic group include tyrosine (“Tyr”), phenylalanine (“Phe”), histidine (“His”), and tryptophan (“Trp”). The penultimate C-terminal “Lip” residue is an amino acid residue that includes a lipophilic alkyl side chain group. The α -carboxyl group of the C-terminal amino acid residue of the present peptides is typically in the form of a carboxylic acid ($-\text{CO}_2\text{H}$). In a preferred embodiment of the invention, the “Lip” and “Ar” residues

are L-amino acid residues. The following standard single letter code abbreviations are used to designate the amino acid residues in the peptides: A - alanine, C - cysteine, D - aspartate, E - glutamate, F - phenylalanine, G - glycine, H - histidine, I - isoleucine, K - lysine, L - leucine, M - methionine, N - asparagine, P - proline, Q - glutamine, R - arginine, S - serine, T - threonine, V - valine, W - tryptophan, Y - tyrosine.

Examples of amino acid residues that have a lipophilic alkyl side chain group include leucine ("Leu"), isoleucine ("Ile"), and valine ("Val"). Typically, the lipophilic alkyl side chain group has a SCDC (cyclohexane-water side chain distribution coefficient calculated as $-RT \ln K_D$ and expressed in kcal/mol) of at least about 3.0 and, preferably, at least about 4.0. For the purposes of this application, SCDC is defined according to Radzicka et al., Biochemistry, 27, 1664 (1988). Where the SCDC of a particular alkyl side chain group is not known, the SCDC value may be determined by measurement of the distribution coefficient between wet cyclohexane and water or by a comparison of a compound containing the same alkyl side chain group with other similar compounds using a hydrophobicity scale derived from HPLC retention according to the method of Parker et al., Biochemistry, 25, 5425 (1986). Despite its similarity in some respects to lipophilic alkyl side chain groups such as leucine, isoleucine, and valine, insertion of a methionine residue at the penultimate position (i.e., an "MY" C-terminal motif) resulted in an inactive analog.

Suitable peptides with a C-terminal Lip-Ar motif for use in the methods of the present invention include WQPPRARIY (SEQ ID NO:1). The alanine knockout analogs (i.e., an analog of a peptide in which a single residue has been substituted by an alanine residue) of the latter peptide that preserve the C-terminal LipAr motif (i.e., retain the C-terminal Ile-Tyr dipeptide sequence) can also be used in the methods of the present invention. Two of the alanine knockout analogs of WQPPRARIY (SEQ ID NO:1) have an alanine residue substituted for one of the arginine residues in the "PRARI" motif (Pro-Arg-Ala-Arg-Ile (SEQ ID NO:15)). These alanine knockout analogs have the amino acid sequences WQPPRAAIY

(SEQ ID NO:2) and WQPPAARIY (SEQ ID NO:4). Two of the other alanine knockout analogs, AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), also differ from WQPPRARIY (SEQ ID NO:1) by a non-conservative amino acid substitution (Ala for Trp and Ala for Gln respectively).

5 Peptides that differ from WQPPRARIY (SEQ ID NO:1) by a non-conservative amino acid substitution but retain the C-terminal LipAr motif can be capable of modulating β 1 integrin subunit dependent cell adhesion even if the overall physical properties of the peptide differ substantially from WQPPRARIY (SEQ ID NO:1). For example, an analog of this peptide in which the two arginine
10 residues have been replaced by aspartic acid residues is suitable for use in the methods of the present invention, as is WQPPDADIY (SEQ ID NO:7), which has an overall net charge of -2 (in contrast to the +2 net charge of WQPPRARIY (SEQ ID NO:1)).

 For certain preferred embodiments of the methods of the present invention,
15 other suitable peptides include those that contain no more than 10 amino acid residues and have a sequence that does not correspond substantially to the amino acid sequence of WQPPRARIY (SEQ ID NO:1). As used herein, the sequence of a particular peptide does not correspond substantially to a reference amino acid sequence, if the particular peptide sequence has less than about 80% identity and
20 preferably less than about 50% homology with the reference sequence. As used herein, the term "% homology" refers to the percentage of amino acid residues of a peptide which are either identical to that of an original peptide sequence or differ from the original peptide sequence solely as a result of a conservative amino acid substitution. For example, the peptide PAIFDRSCGS (SEQ ID NO:17) has 40%
25 identity and 80% homology with respect to the peptide sequence PKVMERTCDS (SEQ ID NO:18).

 Even peptides with less than 50% homology with the corresponding C-terminal portion of WQPPRARIY (SEQ ID NO:1) exhibit the capability of inhibiting β 1 integrin subunit dependent adhesion, and thus are suitable for use in
30 the methods of the present invention. Examples of such peptides include

ARITGYIY (SEQ ID NO:8), RARITGYIY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), and RPAPQRWTY (SEQ ID NO:11). One group of particularly suitable peptides of the invention are those which include a C-terminal "IY" motif, i.e., the sequence of the three C-terminal most amino acid residues is Ile-Ile-Tyr. One such peptide contains 9 amino acid residues and has the sequence ARITGYIY (SEQ ID NO:8). Another group of particularly advantageous peptides of the invention include the C-terminal IY motif and contain no more than ten and, preferably, no more than six amino acid residues. In addition to the dipeptide Ile-Tyr, suitable examples of this group include PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.

For the purposes of this invention, conservative amino acid substitutions are defined to result from exchange of amino acids residues from within one of the following classes of residues: Class I: Ala, Gly, Ser, Thr, and Pro (representing small aliphatic side chains and hydroxyl group side chains); Class II: Cys, Ser, Thr and Tyr (representing side chains including an -OH or -SH group); Class III: Glu, Asp, Asn and Gln (carboxyl group containing side chains); Class IV: His, Arg and Lys (representing basic side chains); Class V: Ile, Val, Leu, Phe and Met (representing hydrophobic side chains); and Class VI: Phe, Trp, Tyr and His (representing aromatic side chains). The classes also include related amino acids such as 3Hyp and 4Hyp in Class I; homocysteine in Class II; 2-aminoadipic acid, 2-aminopimelic acid, γ -carboxyglutamic acid, β -carboxyaspartic acid, and the corresponding amino acid amides in Class III; ornithine, homoarginine, N-methyl lysine, dimethyl lysine, trimethyl lysine, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, homoarginine, sarcosine and hydroxylysine in Class IV; substituted phenylalanines, norleucine, norvaline, 2-aminooctanoic acid, 2-aminoheptanoic acid, statine and β -valine in Class V; and naphthylalanines, substituted phenylalanines, tetrahydroisoquinoline-3-carboxylic acid, and halogenated tyrosines in Class VI.

The peptides of the invention may be synthesized by the solid phase method using standard methods based on either t-butyloxycarbonyl (BOC) or 9-

fluorenylmethoxy-carbonyl (Fmoc) protecting groups. This methodology is described by G.B. Fields et al. in Synthetic Peptides: A User's Guide, W.M. Freyman & Company, New York, NY, pp. 77-183 (1992). The present peptides may also be synthesized via recombinant techniques well known to those skilled in the art. For example, U.S. Patent No. 5,595,887 describes methods of forming a variety of relatively small peptides through expression of a recombinant gene construct coding for a fusion protein which includes a binding protein and one or more copies of the desired target peptide. After expression, the fusion protein is isolated and cleaved using chemical and/or enzymatic methods to produce the desired target peptide.

The peptides used in the methods of the present invention may be employed in a monovalent state (i.e., free peptide or a single peptide fragment coupled to a carrier molecule). The peptides may also be employed as conjugates having more than one (same or different) peptide fragment bound to a single carrier molecule. The carrier may be a biological carrier molecule (e.g., a glycosaminoglycan, a proteoglycan, albumin or the like) or a synthetic polymer (e.g., a polyalkyleneglycol or a synthetic chromatography support). Typically, ovalbumin, human serum albumin, other proteins, polyethylene glycol, or the like are employed as the carrier. Such modifications may increase the apparent affinity and/or change the stability of a peptide. The number of peptide fragments associated with or bound to each carrier can vary, but from about 4 to 8 peptide fragments per carrier molecule are typically obtained under standard coupling conditions.

For instance, peptide/carrier molecule conjugates may be prepared by treating a mixture of peptides and carrier molecules with a coupling agent, such as a carbodiimide. The coupling agent may activate a carboxyl group on either the peptide or the carrier molecule so that the carboxyl group can react with a nucleophile (e.g., an amino or hydroxyl group) on the other member of the peptide/carrier molecule, resulting in the covalent linkage of the peptide and the carrier molecule. Preferably, the conjugate includes at least one peptide fragment which is not linked to the carrier molecule through an amide bond with the α -

carboxyl group of the C-terminal aromatic amino acid residue of the LipAr-terminated fragment.

For example, conjugates of a peptide coupled to ovalbumin may be prepared by dissolving equal amounts of lyophilized peptide and ovalbumin in a small volume of water. In a second tube, 1-ethyl-3-(3-dimethylamino-propyl)-carboiimide hydrochloride (EDC; ten times the amount of peptide) is dissolved in a small amount of water. The EDC solution was added to the peptide/ovalbumin mixture and allowed to react for a number of hours. The mixture may then dialyzed (e.g., into phosphate buffered saline) to obtain a purified solution of peptide/ovalbumin conjugate. Peptide/carrier molecule conjugates prepared by this method typically contain about 4 to 5 peptide fragments per ovalbumin molecule.

As shown in Example 5, the peptides described herein are degraded enzymatically. Thus, to prolong activity of the peptides for any of the methods described herein, a compound that inhibits the enzymatic degradation of the β 1-integrin inhibitor can be coadministered with the β 1-integrin inhibitor.

The present invention also provides a composition that includes one or more active agents (i.e., compound such as a peptide) of the invention and one or more pharmaceutically acceptable carriers. The methods of the invention include administering to a patient, preferably a mammal, and more preferably a human, the composition of the invention in an amount effective to produce the desired effect. The agents of the present invention are formulated in pharmaceutical compositions and then, in accordance with the methods of the invention, administered to a mammal, such as a human patient, in a variety of forms adapted to the chosen route of administration. The formulations include those suitable for oral, rectal, vaginal, topical, nasal, ophthalmic, or parental (including subcutaneous, intramuscular, intraperitoneal, intratumoral, and intravenous) administration. They may be used as a perfusate for organ transplantation. Preferably, the route of administration is topical or intravenous, or as a perfusate for the preparation of organs for transplantation.

The formulations may be conveniently presented in unit dosage form and

may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active agent into association
5 with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulations.

Formulations suitable for parenteral administration conveniently include a sterile aqueous preparation of the active agent, or dispersions of sterile powders of the active agent, which are preferably isotonic with the blood of the recipient.
10 Isotonic agents that can be included in the liquid preparation include sugars, buffers, and sodium chloride. Solutions of the active agent can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions of the active agent can be prepared in water, ethanol, a polyol (such as glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, glycerol esters, and mixtures
15 thereof. The ultimate dosage form is sterile, fluid, and stable under the conditions of manufacture and storage. The necessary fluidity can be achieved, for example, by using liposomes, by employing the appropriate particle size in the case of dispersions, or by using surfactants. Sterilization of a liquid preparation can be achieved by any convenient method that preserves the bioactivity of the active
20 agent, preferably by filter sterilization. Preferred methods for preparing powders include vacuum drying and freeze drying of the sterile injectible solutions. Subsequent microbial contamination can be prevented using various antimicrobial agents, for example, antibacterial, antiviral and antifungal agents including parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Absorption
25 of the active agents over a prolonged period can be achieved by including agents for delaying, for example, aluminum monostearate and gelatin.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as tablets, troches, capsules, lozenges, wafers, or cachets, each containing a predetermined amount of the active agent as a powder or
30 granules, as liposomes containing the chemopreventive agent, or as a solution or

suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion, or a draught. Such compositions and preparations typically contain at least about 0.1 wt-% of the active agent. The amount of active agent is such that the dosage level will be effective to produce the desired result (e.g., suppress the development of cancer or tissue destruction by leukocyte mediated inflammation) in the subject.

Nasal spray formulations include purified aqueous solutions of the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids. Ophthalmic formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye. Topical formulations include the active agent dissolved or suspended in one or more media such as mineral oil, petroleum, polyhydroxy alcohols, or other bases used for topical pharmaceutical formulations.

Useful dosages of the active agents can be determined by comparing their *in vitro* activity and the *in vivo* activity in animals models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art; for example, see U.S. Patent No. 4,938,949. Generally the concentration of the active agent in a liquid composition will be at least about 0.1 wt-% (wt-%, weight percent, means grams of compound per 100 mL liquid). For adult humans, single dosages for intravenous or topical administration will generally be about 0.005 mg to about 50 mg, and may be administered, for example, about 3 to about 5 times per day, to yield levels of about 0.02 mg to about 200 mg per kg of body weight per day. Suitable doses to be administered, in general, are those that are sufficient to produce the desired result. This will typically not exceed 100 micromoles per kg of body weight per day, and may be much lower.

The tablets, troches, pills, capsules, and the like may also contain one or more of the following: a binder such as gum tragacanth, acacia, corn starch or

gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, fructose, lactose or aspartame; and a natural or artificial flavoring agent. When the unit dosage form is a capsule, it may further contain a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac, or sugar and the like. A syrup or elixir may contain one or more of a sweetening agent, a preservative such as methyl- or propylparaben, an agent to retard crystallization of the sugar, an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol, a dye, and flavoring agent. The material used in preparing any unit dosage form is substantially nontoxic in the amounts employed. The active agent may be incorporated into sustained-release preparations and devices.

The invention will be further described by reference to the following detailed examples. The examples are meant to provide illustration and should not be construed as limiting the scope of the present invention.

Examples

EXAMPLE 1

Burn Study

METHODS

New Zealand White rabbits (3-5 kg) (Myrtle's Rabbitry, Thompson Station, TN) were shaved and baseline cutaneous blood flow was measured using a laser Doppler blood flow meter with temperature controlled integrated probe (Perimed PF 4001 Stockholm Sweden) (Johnson, Laser-Doppler blood flowmetry; Sherphard AP, Oberg PA, eds.; Kluwer Academic Publishers, Norwell, MA; pages 121-139, (1990)). The animals were anesthetized using isoflurane inhalation (Abbott Laboratories, North Chicago, IL). Twenty-four gauge catheters (Becton-Dickinson,

Sandy, UT) were placed in ear veins. Three connected brass templates measuring 3 cm x 1 cm x 1 cm each with intervening 5 mm spaces were heated to 100°C and applied to the animals' backs for 30 seconds to create full thickness burns with intervening 5 mm zones of stasis (Mileski et al., J. Surg. Res., 52, 334-9 (1992);
5 Nwariaku et al., J. Surg. Res., 63, 355-8 (1996)). Analgesia with buprenorphine was administered 0.05 mg/kg every 12 hours (Reckitt & Colman Products Ltd., Hull, England). Blood flow measurements were obtained at the burn sites, the zones of stasis surrounding the burn site, and in unburned sites at 24, 48, and 72
10 hours post-burn. The number of zones progressing to necrosis was determined at 72 hours. Animals were killed at 72 hours with intravenous pentobarbital 100 mg/kg (Abbott Laboratories, North Chicago, IL).

Trp-9-Tyr is a nine amino acid synthetic peptide of the fibronectin molecule (Peninsula Laboratories, San Carlos, CA) having the sequence WQPPRARIY (SEQ ID NO:1). It is stored in powder form at 4°C. Immediately prior to use the
15 powder is dissolved in normal saline to a 10 mg/ml solution for intravenous injection.

There were three experimental groups. Controls (n=10) were given saline (1.0 ml/kg). The first treatment group (n=10) was given the peptide, Trp-9-Tyr (5 mg/kg) immediately after burn, then 3, 6, 12, and 24 hours post-burn. The second
20 treatment group (n=10) received Trp-9-Tyr immediately after burn, then every six hours for 48 hours post-burn.

All animal experiments were approved by and performed in accordance with policies of the Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston.

25

RESULTS

There were no significant differences in weight change following burn in the three groups. Gross visual observation and laser doppler blood flow measurement of the burn sites were consistent with full thickness injury. Average baseline laser
30 Doppler blood flows of the three groups ranged from 70.69 to 80.56 perfusion units.

Bloodflow. Control animals had diminished blood flow in the zone of stasis at 24 hours post-burn (29.97 ± 2.94). There was some recovery at 48 and 72 hours. However, blood flow remained significantly lower than baseline blood flow.

Animals in the 24-hour treatment group had significantly higher blood flow in the zone of stasis than controls at 24 hours post-burn (42.25 ± 3.40). However, there was no difference in perfusion at 48 and 72 hours.

Skin perfusion of the 48-hour treated animals was significantly higher than controls and animals treated for 24 hours at all time points measured. Although blood flow was significantly lower than baseline at 24 hours post-burn (56.23 ± 2.55), perfusion recovered and was not significantly different from baseline at either 48 or 72 hours (Figures 1 and 2).

Tissue Necrosis. Control animals had 16 of 40 (40%) of zones of stasis progressing to necrosis at 72 hours. Animals receiving Trp-9-Tyr for 24 hours had 7 of 40 (17%) of zones progressing to necrosis, significantly less tissue destruction than controls ($p < 0.05$ chi-square). Animals treated for 48 hours had 4 of 40 (10%) of zones progressing to necrosis, which was significantly less tissue loss than controls ($p < 0.05$ chi-square, Mann-Whitney) (Table 1 and Figures 8-10).

Table 1
Tabulation of zones of stasis progressing to necrosis comparing the 24- and 48 hour-treatment groups to controls.

| | Number of Animals | Total # Zones | Zones with Necrosis |
|---------|-------------------|---------------|---------------------|
| Control | 10 | 40 | 16 |
| 24 hour | 10 | 40 | 7* |
| 48 hour | 10 | 40 | 4* |

*Animals in the 24-hour treatment group had 7 of 40 zones (17%) progress to necrosis, significantly fewer zones than controls ($p < 0.05$ chi-square). Forty-eight hour treatment animals had necrosis in only 4 of 40 zones (10%), significantly less tissue destruction than controls ($p < 0.05$ chi-square and Mann-Whitney).

DISCUSSION

Emphasis on microvascular injury secondary to leukocyte adherence to the endothelium has been the key to investigation of substances which inhibit inflammation. More recently, the extracellular matrix has become a point of interest for investigation of the inflammatory response to injury. The extracellular matrix macromolecule, fibronectin has reemerged as having a potentially significant role in the pathogenesis of the inflammatory processes.

Synthetic analogues of the RGD domain of fibronectin have been used to prevent acute and chronic experimental liver injury in mice. Animals receiving intravenous RGD mimetics had lower serum levels of liver enzymes and less liver damage by histology than untreated mice following induction of hepatitis from intravenous concanavalin A injection (Bruck et al., Yale J. Biol. Med., 70, 391-402 (1997)).

Synthetic fibronectin peptides derived from the 33-kD carboxyl-terminal heparin-binding domain of fibronectin have demonstrated efficacy in blocking neutrophil accumulation in models of acute inflammation. Two of the more potent synthetic peptides identified thus far are WQPPRARIY (SEQ ID NO:1) and CS-1. Using a rodent model of ischemic brain injury, Yanaka, et al. demonstrated that administration of WQPPRARIY (SEQ ID NO:1) and CS-1 decrease leukocyte accumulation, effectively reduced infarct size, and improved neurological assessment (Yanaka et al., J. Neurosurg., 85, 125-30 (1996); and Yanaka et al., J. Cereb. Blood Flow Metab., 16, 1120-5 (1996)). Synthetic fibronectin peptides block development of inflammatory lesions in salivary glands of TGF- β_1 knock-out mice and restore saliva production (McCartney-Francis et al., J. Immunol., 157:1306-12 (1996)). These peptides blocked leukocyte infiltration into heart and lung tissues of TGF- β_1 knock-out mice, as demonstrated by histopathology. These animals had reduced weight loss and extended life span compared to untreated knock-out mice (Hines et al., Proc. Natl. Acad. Sci., USA, 91, 5187-91 (1994)).

As a cationic hydrophilic peptide, WQPPRARIY (SEQ ID NO:1) is thought to adhere to cell surface proteoglycans (Woods et al., Mol. Biol. Cell., 4, 605-13 (1993); and Wahl et al., J. Clin. Invest., 94, 655-62 (1994)). Cell surface

proteoglycans, which mediate a spectrum of cell-binding activities may alter proteoglycan interaction with selectins and/or integrin-dependent leukocyte homing (Woods et al., Mol. Biol. Cell., **4**, 605-13 (1993); Hines et al., Proc. Natl. Acad. Sci., USA, **91**, 5187-91 (1994); and Iida et al., J. Cell Biol., **118**, 431-44 (1992)).

5 Migration of neutrophils into sites of inflammation has been reported to be initiated by a factor released by monocytes (Mileski et al., Circ. Shock, **31**, 259-67 (1990)). The synthetic fibronectin peptide, Trp-9-Tyr, has also been demonstrated to inhibit monocyte accumulation via the pathways related to CS-1 (Wahl et al., J. Clin. Invest., **94**, 655-62 (1994)). CS-1 interacts with $\alpha_4\beta_1$ integrin expressed on
10 monocytes, altering their function. This suggests that the synthetic fibronectin peptides may inhibit late accumulation of neutrophils into inflammatory tissue.

Other mechanisms of action for the peptide WQPPRARIY (SEQ ID NO:1) have been considered. It may block signal transduction pathways and cytokine presentation. In addition, an $\alpha_4\beta_1$ integrin also serves as a receptor for vascular cell
15 adhesion molecule-1 (VCAM-1), which is expressed on endothelial cells (Wahl et al., J. Clin. Invest., **94**, 655-62 (1994)). By interacting with $\alpha_4\beta_1$ integrin, the peptide WQPPRARIY (SEQ ID NO:1) may block leukocyte adhesion to the microvascular endothelium.

In this study, the administration of synthetic fibronectin peptide, Trp-9-Tyr
20 (WQPPRARIY (SEQ ID NO:1)), reduced tissue necrosis following burn injury. These results were somewhat evidenced in the 24-hour treatment group. However, the improvements in blood flow and tissue necrosis were significant in the 48-hour treatment group.

We hypothesize that the difference in blood flow seen at 24 hours between
25 the two treatment groups was due to variation in the dosing schedules. Twenty-hour treatment animals received Trp-9-Tyr immediately, then 3, 6, 12, and 24 hours. The 48-hour treatment group received an 18-hour dose rather than a 3-hour dose. It is possible that the constant 6-hour dosing interval is more effective than an initial bolus of Trp-9-Tyr. Furthermore, the inflammatory process produces tissue
30 destruction for a period longer than 24 hours. By extending the treatment beyond

METHODS

New Zealand white rabbits (1.8-2.5 kg each) were the experimental subjects. Animals were prepared by insertion of a 24-gauge catheter in a marginal ear vein, and clipping of the hair on their dorsa.

Baseline weights, temperatures, hematocrits and WBC counts were obtained. Then the animals were given two sets of paired subcutaneous injections with *S. aureus* ATCC 25923. Following these injections animals were given Trp-9-Tyr as described below. In addition animals were given the antibiotic cefazolin (20 mg/kg intravenously every 8 hours for three doses). Weights, temperature, WBC counts and hematocrits were measured daily for 7 days. Buprenorphine (0.05 mg/kg) was administered for analgesia as determined from a quantitative assessment of pain score. The animals were sacrificed with a lethal intravenous injection of pentobarbital (150 mg/kg) on day 7, and the dorsal skin was dissected from the muscular fascia to allow determination of the incidence of subcutaneous abscess formation and measurement of abscess size by planimetry.

Standard clinical laboratory *S. aureus* ATCC 25923 was grown on tryptic soy agar blood plates at 37°C, harvested and resuspended in sterile saline at concentrations of 10^8 and 10^9 CFU/ml as determined by A540 measurements. The animals were given two subcutaneous injections (1.0 ml each) of the 10^9 CFU suspension and two subcutaneous injections (1.0 ml each) of the 10^8 CFU suspension via a 25-gauge needle and 3.0 ml syringe. Quantitative correlations of bacterial inocula were performed on each day of bacterial preparation. Mean CFU of *S. aureus* were 1.02 ± 0.21 times the expected concentrations determined by optical density. Statistical analysis of the data was performed by analysis of variance for repeated measures, t test, chi-square test and Kaplan-Meier survival analysis. Significance was assigned to $P < 0.05$.

Peptide Administration. Trp-9-Tyr synthetic peptide (stored in powder form at 4°C) was dissolved in normal saline to a 10 mg/ml solution for intravenous injection immediately before use. There were two treatment groups, control and

peptide treatment. Controls were given saline (1.0 ml/kg). Treatment group was given the peptide, Trp-9-Tyr (5 mg/kg) immediately after burn, then 3, 6, 12, 24 and 48 hours post-burn.

All animal experiments were approved by and performed in accordance with policies of the Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston.

RESULTS

The results of these studies are shown in Figures 2-7, 11, and 12. The number of abscesses formed in treated animals was not greater than that of control animals that did not receive the $\beta 1$ -integrin inhibitory peptide (Figure 11). In addition, the abscess size in treated animals was not greater than that observed in controls; and if anything the abscess size in treated animals was reduced from that observed in controls (Figure 12).

Daily measurements of temperature, body temperature, WBC and hematocrit (Figures 4-7, respectively) were not elevated compared to control animals, as would have been expected if treatment with $\beta 1$ -integrin inhibitory peptide Trp-9-Tyr had blocked the ability to response to bacterial infection. In contrast, daily temperature measurements for peptide treated animals were actually less than controls. In addition, treated animals not only had a similar number and size of abscess formation, but also had significantly better weight gain profile than controls (Figure 3). In conclusion, this study found no evidence that (Trp-9-Tyr) possesses immuno-comprising activity.

EXAMPLE 3

Local Infusion of Two Peptides in Stroke Model

MATERIALS AND METHODS

Adult Sprague-Dawley rats, weighing 300-350 grams, were randomly assigned to three groups: phosphate buffered saline (PBS) (n=6), PRARI (SEQ ID NO:15) (n=4), and PRARIY (SEQ ID NO:12) (n=5). The rats were subjected to 1 hour MCAO (middle cerebral artery occlusion) with 48 hours reperfusion. The

peptides, PRARI (SEQ ID NO:15) and PRARIY (SEQ ID NO:12) (20 mg/kg in PBS), and the same volume of PBS were continuously infused through extra common artery (local infusion) by an osmotic minipump (0.8 μ l/hour) at the time of reperfusion. Neurological deficits were tested at 3, 24, and 48 hours after MCAO, and marked with a grading scale of 0-5 (0, no neurological deficits; 5, severe neurological deficits). Forty-eight hours after reperfusion, the rats were sacrificed and the brains were quickly removed and frozen in powdered dry ice. The coronal cryostat sections, 20 μ m thickness, 900 μ m interval were used for infarction size measurements (data shown in Figure 13); and 10 μ m thick sections through infarction area were taken for immunohistochemical staining to detect leukocyte infiltration (by MPO antibody). Neurological analysis is presented in Figure 14.

RESULTS

The peptide PRARIY (SEQ. ID NO:12) treated rats demonstrated a significant functional improvement at 24 hours and 48 hours after MCAO in comparison with PBS-treated rats; no significant difference between PRARI (SEQ ID NO:15) and PBS, PRARI (SEQ ID NO:15) and PRARIY (SEQ ID NO:12) treated groups was observed. The infarction volume was significantly reduced in PRARIY (SEQ ID NO:12) treated rats when compared with PBS group; no significant difference between PRARI (SEQ ID NO:15) versus PBS and PRARI (SEQ ID NO:15) versus PRARIY (SEQ ID NO:12) treated groups was observed. There was no difference in leukocyte recruitment among the three groups.

DISCUSSION

This experiment was conducted to determine the anti-adhesion effect of the peptide PRARIY (SEQ ID NO:12) administered via local infusion on brain ischemia/reperfusion injury. The results show that the peptide PRARIY (SEQ ID NO:12) protects neurons against brain ischemia/reperfusion injury as evidenced

by the smaller infarct size and the rapid reversal of transient neurological deficit. With local infusion of the peptide near the site of injury, at least 80% reduction in infarct size and at least 80% reduction in neurological deficit was observed.

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EXAMPLE 4

Equivalence of β 1-integrin Inhibitor Peptide to Anti- β 1-integrin Antibody

Soluble peptides or specific anti- β 1-integrin antibody was mixed with lymphocytes prior to the start of the adhesion assay. Following a brief (15 minute) preincubation, the cells were added to culture wells containing confluent endothelial that had been activated with cytokines. Following a 30 minute incubation, the lymphocyte/endothelial cultures were washed to remove weakly and nonadherent cells. The data represent the percentage of input cells remaining after this washing step. See Figures 15 and 16. The results indicate that either Trp-9-Tyr or a specific inhibitory anti-integrin antibody can achieve the same level of inhibition of adhesion in this assay.

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EXAMPLE 5

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Stability and Pharmacokinetics of a Bioactive Peptide

METHODS

Stability Studies In Vitro Stability studies were performed in various media, including aqueous solution, rat plasma, human plasma, plasma treated with methanol and rat blood. Solutions of WQPPRARIY (SEQ ID NO:1) (concentration range, 10-400 mcg/ml) were incubated at 37 °C for periods up to 4 hours. Aliquots of the plasma incubates, taken frequently at early times, were immediately added to methanol to deactivate enzymes, diluted with water and applied to C-2 cartridges for solid-phase extraction of WQPPRARIY (SEQ ID NO:1). After clean up and elution, aliquots of the eluant were analyzed by a validated high-performance liquid chromatographic assay with an UV detector at wavelength of 220 nm following reversed-phase separation on a C-18 column (4.6*100 mm). The mobile phase was ammonium acetate (0.05 M, pH 4.53):

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methanol (58:42 v/v) and flow rate was 0.5 ml/minute. Plots of peak area (or height) ratio to internal standard (PX7068) vs. WQPPRARIY (SEQ ID NO:1) concentration were linear from 2-400 mcg/ml in human plasma, rat plasma and rat blood. Aqueous solution samples were directly injected onto the column after incubation.

Pharmacokinetic Study Four rats (225-280 g) were used in the pharmacokinetic study. The animal protocol was reviewed and approved prior to animal experimentation by the University of Minnesota's committee on animal use and care. The femoral artery and vein were cannulated for drug sampling and administration, respectively. This was a two-phase crossover study. The first phase was a low-dose (5 mg/min-kg) infusion for 15 minutes. Immediately after this, the animals received a high-dose (10 mg/kg/min) for 15 minutes, followed by a washout period. Intravenous formulations of WQPPRARIY (SEQ ID NO:1) were prepared by dissolving an accurately weighed amount of the peptide in physiological saline. Blood samples were collected from femoral artery at 6, 9, 12 and 15 min after dose initiation in both phases. Additionally, from 3 to 6 samples were drawn in the 5 minutes following the termination of the high infusion rate. Blood samples were immediately treated with methanol to inactivate enzymes, and processed using the same procedure described for the plasma study performed *in vitro*.

The blood samples from the pharmacokinetic study were analyzed by a validated high-performance liquid chromatographic assay with an UV detector at wavelength of 220 nm. Mobile phase was as described above. Within-run and between-run variability of the assay was characterized by CVs of less than 10%. The average analytical recovery was $70 \pm 4.2\%$. Similar results were found in rat plasma and rat blood. The inclusion of quality control samples demonstrated accuracy, precision, and reproducibility of the method.

RESULTS

The peptide WQPPRARIY (SEQ ID NO:1) was stable in PBS (0.1 M, pH 7.4) and in water over 4 hours of incubation at 37°C but markedly unstable in rat plasma and human plasma. At initial concentrations of 10 to 40 mcg/ml in human plasma the peptide exhibits an effective half-life of less than 3 minutes; at 100 and 400 mcg/ml it exhibited half-lives of 5 minutes and 15 minutes, respectively (Figure 17).

Although not plotted here, similar degradation rates were seen in rat plasma. Chromatographic peaks of several degradation products were observed in both human and rat plasma and these exhibited longer half lives than WQPPRARIY (SEQ ID NO:1). In plasma treated with methanol, the peptide was stable over the 4-hour incubation at 37°C.

The blood concentration-time data obtained in the constant-rate infusion studies were subjected to compartment analysis using SAAM II (Program for Kinetic Analysis, version 1.1.1). It was assumed that WQPPRARIY (SEQ ID NO:1) obeyed a one-compartment model with one elimination pathway of the Michaelis-Menten type. Parameters determined in the analysis were V (liter), the maximum velocity of metabolism (V_{max}), and K_m . The mean and individual pharmacokinetics parameters are provided in Table 2. The mean plasma concentration versus time profiles of WQPPRARIY (SEQ ID NO:1) during the two-phase infusion study are depicted in Figure 18.

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Table 2. Individual and Mean (SD) Pharmacokinetic Parameters in Rats.

| Parameter | Rat | | | Mean (SD) |
|---------------------------------|------|------|------|-------------|
| | 1 | 2 | 3 | |
| V (L/kg) | 0.54 | 0.71 | 1.07 | 0.77 (0.27) |
| V _{max} (mg/min-kg) | 15.6 | 15.5 | 13.5 | 14.9 (1.2) |
| K _m (mg/L) | 17.5 | 17.9 | 20.9 | 18.8 (1.9) |

5 Based on preliminary studies not reported here, the half-life of this peptide *in vivo* was estimated to be less than two minutes in concentrations produced by doses of 10 to 20 mg/kg. The infusion study was performed in order to obtain estimates of clearance at steady state, and to characterize saturability of elimination, a crossover study with two infusion rates was employed.

10 Blood concentrations of WQPPRARIY (SEQ ID NO:1) approached steady state during infusion period, and mean blood concentration (SD) during the low and high dose phases were 11.4 (0.84) and 36.33 (4.0) µg/ml, respectively. This lack of proportionality between steady-state concentrations and infusion rates is a characteristic of Michaelis-Menten elimination kinetics, and is consistent with the results of the *in vitro* studies. Parameters are calculated using SAAM II in three rats. The mean apparent volume of distribution (SD) is 0.77 (0.27) L, which is

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similar to the volume of total body water in the rat, suggesting significant distribution of the peptide into tissues.

It should be noted that if two sets of steady-state blood concentrations at two infusion rates are available, one can normally estimate the operative Michaelis-Menten parameters that describe the saturability of metabolism of the infused therapeutic agent. In order to estimate a volume of distribution, changing blood levels within the animal need to be determined, a challenge for a compound with a short effective half-life. Thus, data from one of the four animals, which afforded only 2 measurable samples in the post-infusion period, did not allow convergence in SAAM II and are not included in the statistical analysis and parameter summary.

The short half-life of WQPPRARIY (SEQ ID NO:1) in plasma *in vitro* demonstrates rapid enzymatic degradation that does not require perfusion of the peptide through organ systems in order for metabolism to occur. It is of interest that when the half-life of the peptide is calculated from means of the estimates of the Michaelis-Menten parameters and the volume of distribution, these are found to range from 1.1 to 15.5 min for concentrations ranging from 10 to 400 mg/Liter. These values are consistent with those found in the studies *in vitro*, and seem to suggest that circulating enzymes are primarily responsible for the disposition of FN-C/H-V.

Finally, a pharmacokinetic study of the rapidly metabolized peptide WQPPRARIY (SEQ ID NO:1) was performed *in vivo* using high dose-short infusion periods at two rates. Michaelis-Menten parameters, suggesting saturation of blood-borne peptidases were estimated. The results are consistent with the degradation of the peptide incubated in rat and human plasma, and suggest efficient processing by peptidases. Although not identified, intermediates that resulted from the degradation of WQPPRARIY (SEQ ID NO:1) were observed in the studies *in vitro*. However, their role in inhibiting the cell adhesion ascribed to the parent peptide is unknown.

DISCUSSION

Transient cerebral ischemia and associated brain injury may be mediated by several factors, including inflammatory processes (Hallenbeck et al., Stroke, 17, 246-253 (1986)). Leukocyte infiltration into ischemic tissue is a

5 pathophysiological response, which often further aggravates ischemic injury by attenuating microvascular blood flow, and releasing chemical mediators such as free oxygen radicals (Kochanek et al., Stroke, 23, 1367-1379 (1992); and Matsuo et al., J. Cereb. Blood Flow Met., 15, 941-947 (1995)). Cell adhesion molecules play important roles in leukocyte-endothelial interactions: the selectins

10 (Lasky, Science, 258, 964-969 (1992)), the integrins, and the immunoglobulin superfamilies (Springer, Nature, 346, 425-434 (1990)). Integrins which contain β_1 subunits usually are associated with mediating adhesion to extracellular matrix constituents (Springer, Nature, 346, 425-434 (1990)) whereas β_2 integrins are largely involved in cell-cell interactions. One of these extracellular matrix

15 macromolecules is fibronectin, which is found in plasma, cell matrix, and on the cell surface. These molecules can support leukocyte adhesion to endothelial cells (Akiyama et al., Adv. Enzymol., 57, 1-57 (1987)).

Fibronectin possesses multiple domains recognized by integrins, including arginyl-glycyl-aspartic acid (RGD). The latter interacts selectively with $\alpha_5\beta_1$

20 integrin, and the alternately spliced connecting segment domain (CS-1) which is recognized selectively by $\alpha_4\beta_1$ integrin (Akiyama et al., Adv. Enzymol., 57, 1-57 (1987); and Guan et al., Cell, 60, 53-61 (1990)). Over the last few years several novel (nonRGD/nonCS-1) bioactive peptides from fibronectin that: a) antagonize leukocyte adhesion of activated lymphocytes and monocytes *in vitro* when used as

25 soluble antagonists and b) show efficacy for improved outcomes in several *in vivo* animal models of chronic and acute inflammation when administered intravenously. These models include bacterial cell wall-induced arthritis in rats, models of autoimmune disease such as TGF- β $-/-$ mice, and reperfusion injury in rat transient cerebral ischemia and in rabbit burn models (Hines et al., Proc. Natl. Acad. Sci., USA, 91, 5187-5191 (1994); Wahl et al., J. Clin. Invest., 94, 655-662

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(1994); and unpublished data).

Peptide WQPPRARIY (SEQ ID NO:1) has been characterized over the last several years with regard to structure/activity relationships and its molecular target of this peptide. The studies have shown that this peptide inhibits adhesion by a unique molecular mechanism. The minimum structure of the peptide (WQPPRARIY (SEQ ID NO:1)) needed to inhibit adhesion has been identified as the last two residues of this peptide (IY) (International Publication No. WO 99/37669). Secondly, this dipeptide has been shown to represent a more generalized structural motif that can effectively inhibit adhesion, where the amino acids consist of a branched hydrophobic residue plus an aromatic residue). Finally, we have demonstrated by several approaches that this structural motif represents a novel peptide based generalized inhibitor of β_1 integrin mediated cell adhesion.

Sequence Free Text

The following are all peptides:

WQPPRARIY (SEQ ID NO:1)
WQPPRAAIY (SEQ ID NO:2)
QPPRAAIY (SEQ ID NO:3)
WQPPAARIY (SEQ ID NO:4)
AQPPRARIY (SEQ ID NO:5)
WAPPRARIY (SEQ ID NO:6)
WQPPDADIY (SEQ ID NO:7)
ARITGYIY (SEQ ID NO:8)
RARITGYIY (SEQ ID NO:9)
PRQAWRPIY (SEQ ID NO:10)
RPAPQRWIY (SEQ ID NO:11)
PRARIY (SEQ ID NO:12)
RARIY (SEQ ID NO:13)
ARIY (SEQ ID NO:14)
PRARI (SEQ ID NO:15)
RPQIPWARY (SEQ ID NO:16)
PAIFDRSCGS (SEQ ID NO:17)
PKVMERTCDS (SEQ ID NO:18)

WHAT IS CLAIMED IS:

1. A method of inhibiting inflammatory leukocyte mediated destruction of tissue in a patient, the method comprising administering to the patient a composition comprising a β 1-integrin inhibitor.
2. The method of claim 1 wherein the β 1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.
3. The method of claim 2 wherein the β 1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAAAIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYIY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWTY (SEQ ID NO:11), PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.
4. The method of claim 1 wherein the composition further includes a pharmaceutically acceptable carrier.
5. The method of claim 1 wherein the inflammatory leukocyte mediated destruction of tissue occurs as a result of CNS ischemic injury, myocardial infarction, angioplasty, surgical incisions, injury-related trauma, transplant reperfusion, or a combination thereof.
6. The method of claim 1 wherein the inflammatory leukocyte mediated destruction of tissue occurs as a result of exposure to heat, cold, light, electricity, chemicals, or a combination thereof.

7. A method of treating a stroke patient, the method comprising administering to the patient a composition comprising a β 1-integrin inhibitor in an amount effective to reduce infarct size, reduce neurological deficit, or both.
8. The method of claim 7 wherein the composition is administered locally.
9. The method of claim 8 wherein the β 1-integrin inhibitor is administered in an amount effective to reduce the infarct size by at least about 80%.
10. The method of claim 8 wherein the β 1-integrin inhibitor is administered in an amount effective to reduce the neurological deficits by at least about 80%.
11. The method of claim 7 wherein the β 1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.
12. The method of claim 11 wherein the β 1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAARIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYTY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWIY (SEQ ID NO:11), PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.
13. A method of treating a patient having a burn-type injury, the method comprising administering a composition comprising a β 1-integrin inhibitor in an amount effective and over a period of time effective to reduce leukocyte-mediated tissue destruction.

14. The method of claim 13 wherein the β 1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.
15. The method of claim 14 wherein the β 1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAARIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYTY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWIY (SEQ ID NO:11), PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.
16. The method of claim 15 wherein the β 1-integrin inhibitor is a peptide comprising the amino acid sequence WQPPRARIY (SEQ ID NO:1).
17. The method of claim 13 wherein the period of time is at least 1 hour.
18. The method of claim 17 wherein the period of time is at least 24 hours.
19. The method of claim 18 wherein the period of time is at least 48 hours.
20. The method of claim 13 wherein the composition is administered periodically over a predetermined period of time.
21. A method of treating a burn patient, the method comprising maintaining a composition comprising an effective amount of a β 1-integrin inhibitor on a burn-type injury for a period of time effective to reduce leukocyte-mediated tissue destruction and achieve a desired degree of healing.

22. The method of claim 21 wherein the β 1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.
23. The method of claim 22 wherein the β 1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAARIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYIY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWIY (SEQ ID NO:11), PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.
24. The method of claim 23 wherein the β 1-integrin inhibitor is a peptide comprising the amino acid sequence WQPPRARIY (SEQ ID NO:1).
25. A method of treating a cancer patient, the method comprising administering to the patient a composition comprising a β 1-integrin inhibitor in an amount effective to inhibit one or more of angiogenesis, cancer cell metastasis, cancer cell motility, or cancer cell migration.
26. The method of claim 25 wherein the β 1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.
27. The method of claim 26 wherein the β 1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAARIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYIY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWIY (SEQ ID NO:11), PRARIY

(SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.

28. A method of treating a cancer patient, the method comprising administering to the patient a composition comprising a β 1-integrin inhibitor in an amount effective to induce programmed cell death in cancerous tissue or restore normal cellular phenotype to cancerous tissue.
29. The method of claim 28 wherein the β 1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.
30. The method of claim 29 wherein the β 1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAARIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYIY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWTY (SEQ ID NO:11), PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.
31. The method of claim 28 further comprising administering a compound that inhibits the enzymatic degradation of the β 1-integrin inhibitor.
32. A method of treating a patient for osteoporosis, the method comprising administering to the patient a composition comprising a β 1-integrin inhibitor in an amount effective to inhibit osteoclast adhesion and bone resorption.
33. The method of claim 32 wherein the β 1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

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34. The method of claim 33 wherein the β 1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAARIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYTY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWIY (SEQ ID NO:11), PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.
35. A method of peripheralizing stem cells, the method comprising administering to a patient a composition comprising a β 1-integrin inhibitor.
36. The method of claim 35 wherein the β 1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.
37. The method of claim 36 wherein the β 1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAARIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYTY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWIY (SEQ ID NO:11), PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.
38. A composition comprising β 1-integrin inhibitor and a pharmaceutically acceptable carrier.
39. The composition of claim 38 wherein the β 1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

40. The composition of claim 39 wherein the β 1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:1), WQPPRAAIY (SEQ ID NO:2), QPPRAAIY (SEQ ID NO:3), WQPPAARIY (SEQ ID NO:4), AQPPRARIY (SEQ ID NO:5), WAPPRARIY (SEQ ID NO:6), WQPPDADIY (SEQ ID NO:7), ARITGYIY (SEQ ID NO:8), RARITGYTY (SEQ ID NO:9), PRQAWRPIY (SEQ ID NO:10), RPAPQRWIY (SEQ ID NO:11), PRARIY (SEQ ID NO:12), RARIY (SEQ ID NO:13), ARIY (SEQ ID NO:14), and RIY.

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(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

(88) Date of publication of the international search report:
31 May 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS OF USE OF BETA 1-INTEGRIN INHIBITORS

(57) Abstract: The present invention provides methods that use agents capable of one or more of the following: inhibiting leukocyte mediated destruction of tissues, especially after injuries to tissue due to heat or cold (i.e., burns or frostbite), light, electricity, chemicals, or other agents that cause tissue injury and destruction; inhibiting leukocyte mediated destruction of tissues after myocardial infarction, CNS ischemic injury (e.g., stroke), angioplasty, surgical incisions, injury-mediated trauma, and transplant reperfusion; inhibiting angiogenesis; inhibiting cancer cell metastasis, motility, and/or migration within tissue; restoring and/or potentiating the induction of programmed cell death in cancerous tissues or restoring normal cellular phenotype to cancerous tissue; inhibiting osteoclast adhesion and bone resorption; and/or inhibiting stem cell adhesion to bone marrow.

WO 00/56350 A3

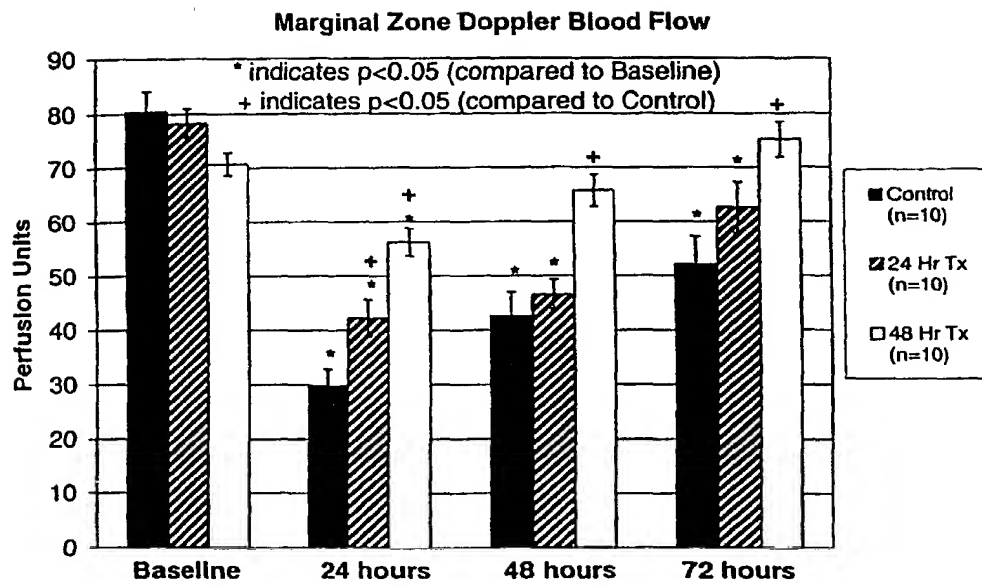


FIG. 1

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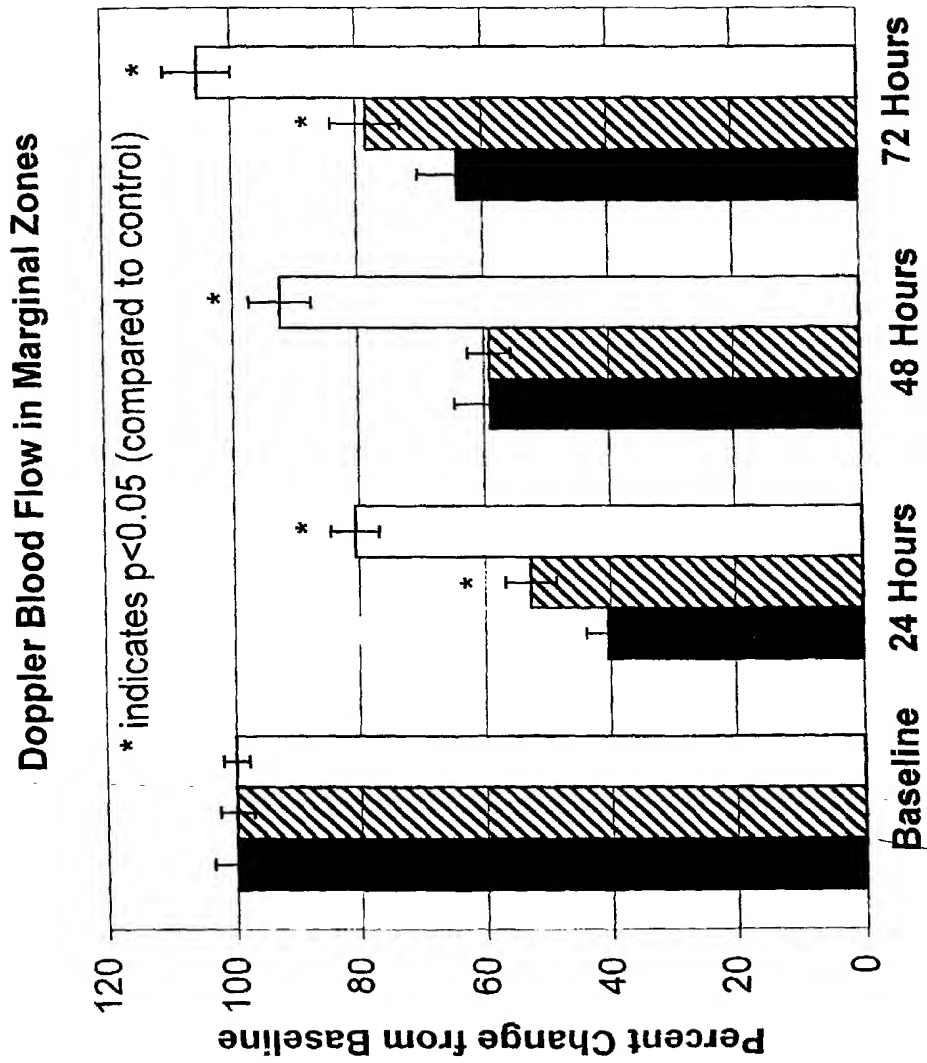


FIG. 2

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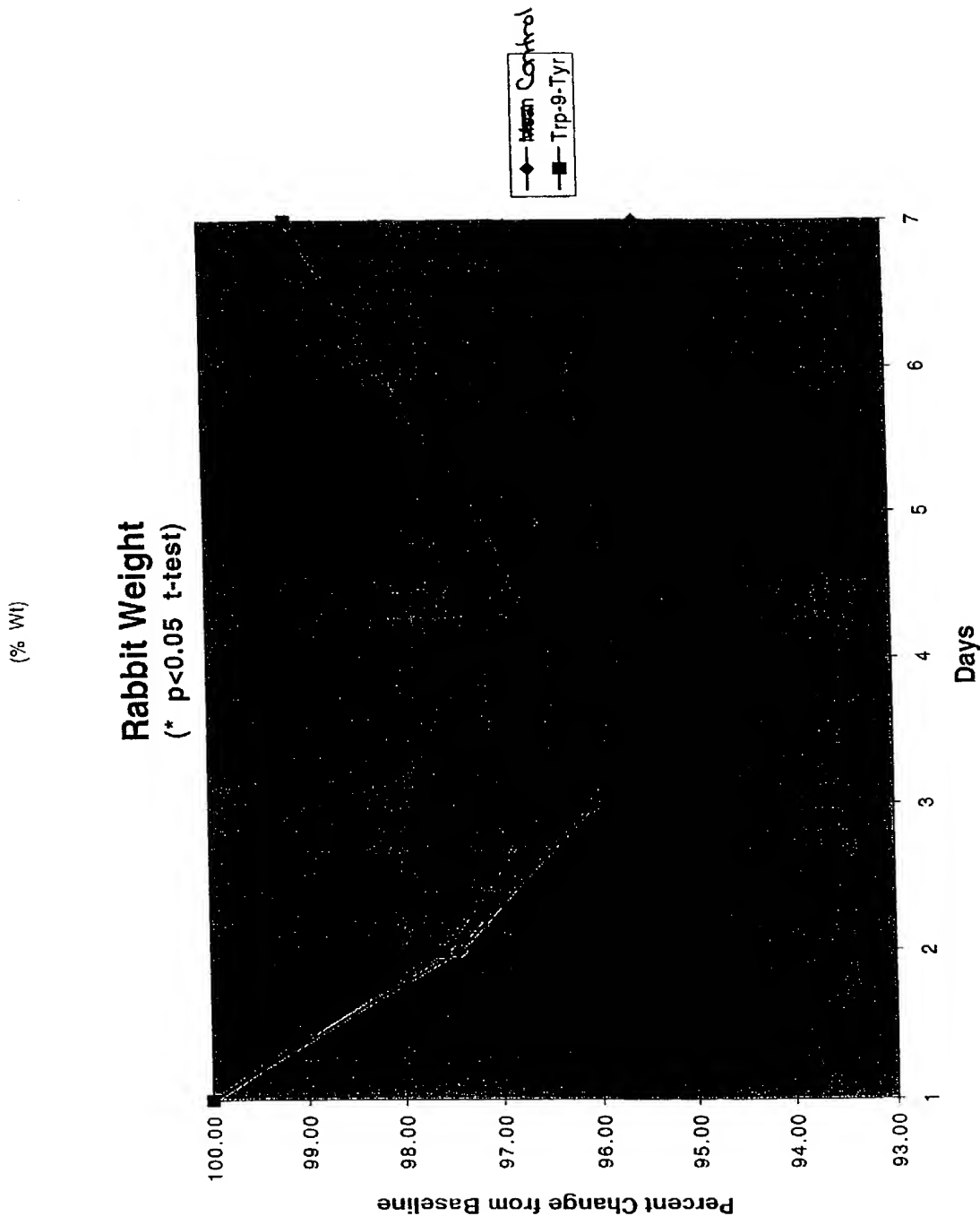


FIG. 3

(Temp)

Daily Temperature (* p<0.05 t-test)

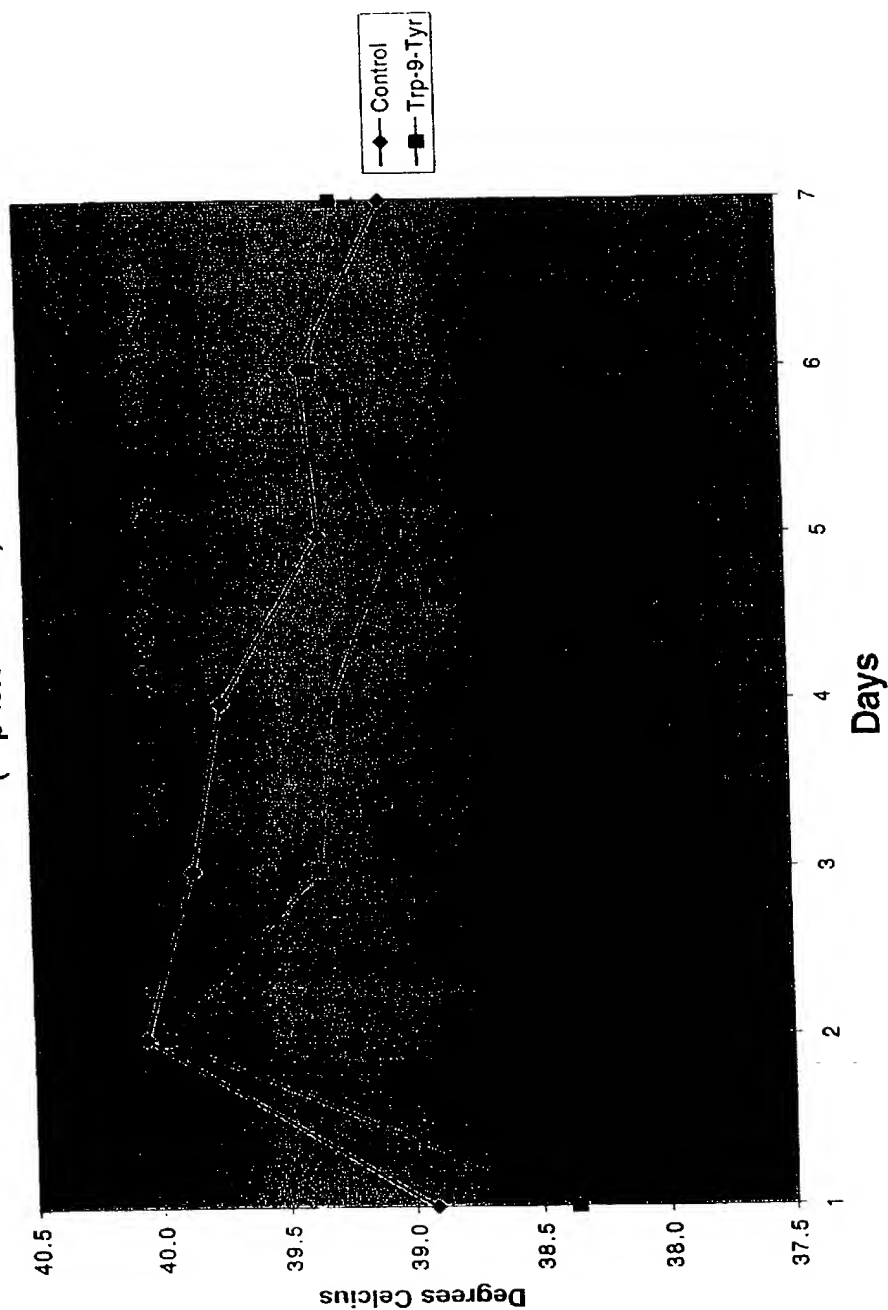


FIG. 4

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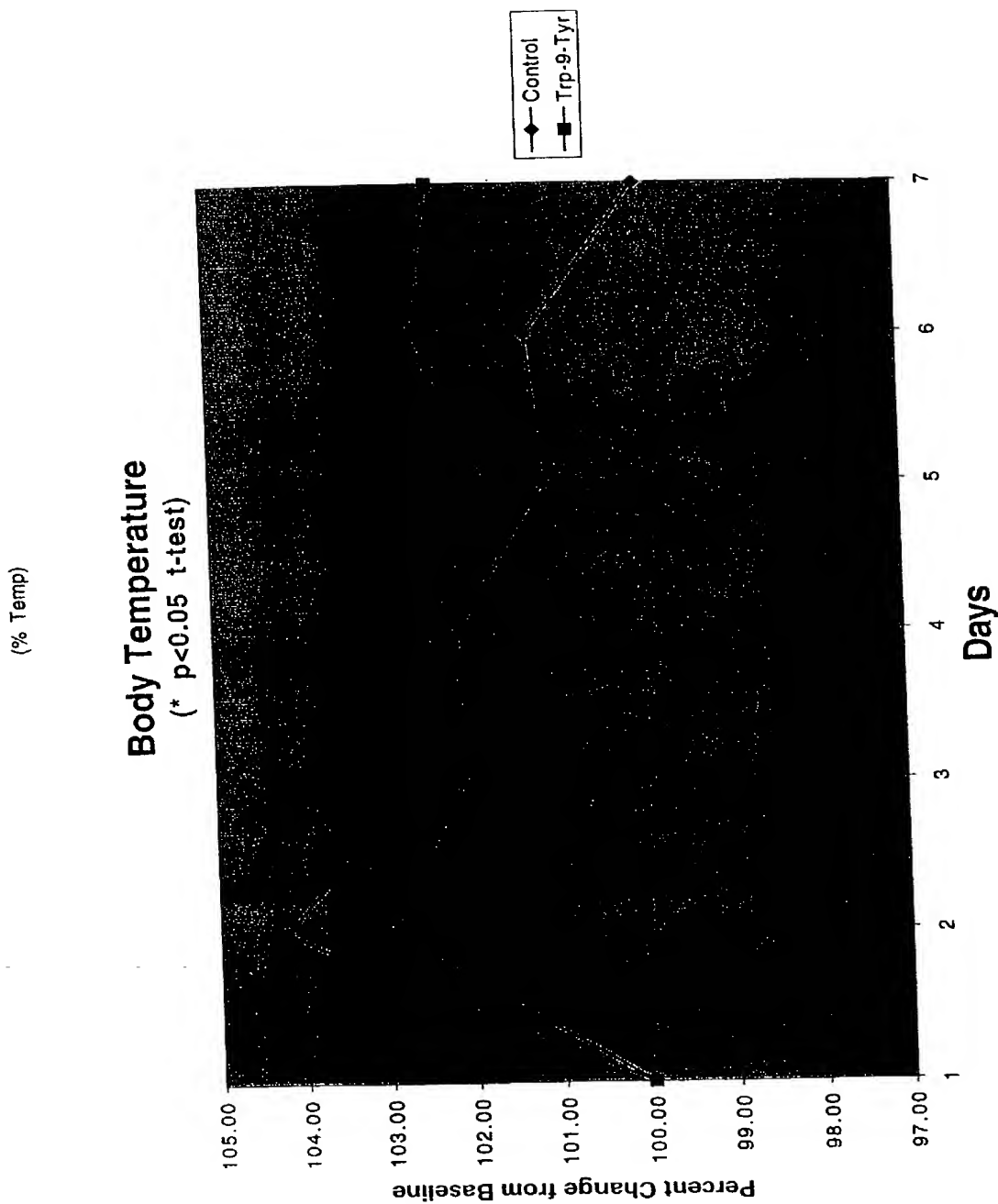


FIG. 5

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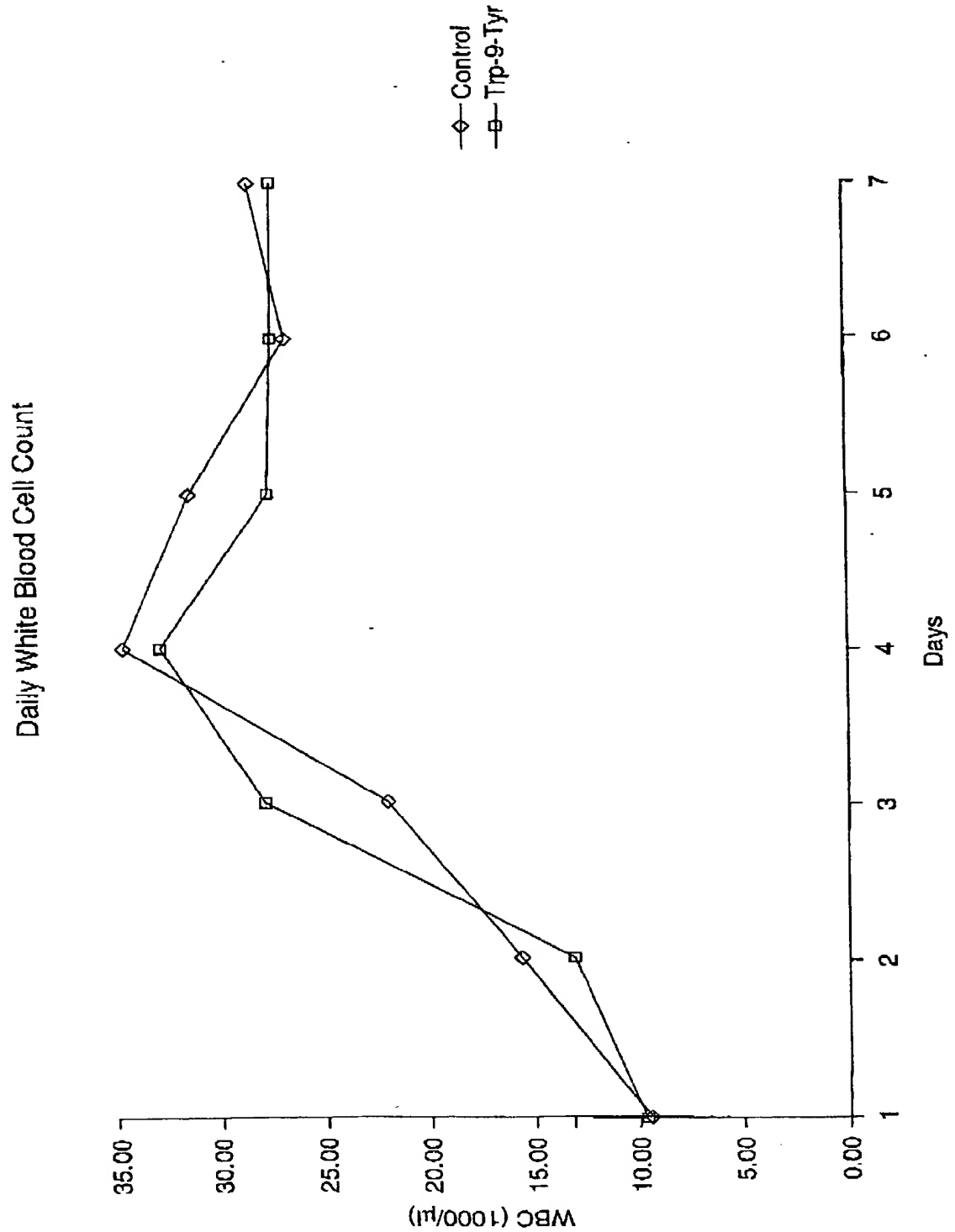


Fig. 6

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(Hct)

Daily Hematocrit

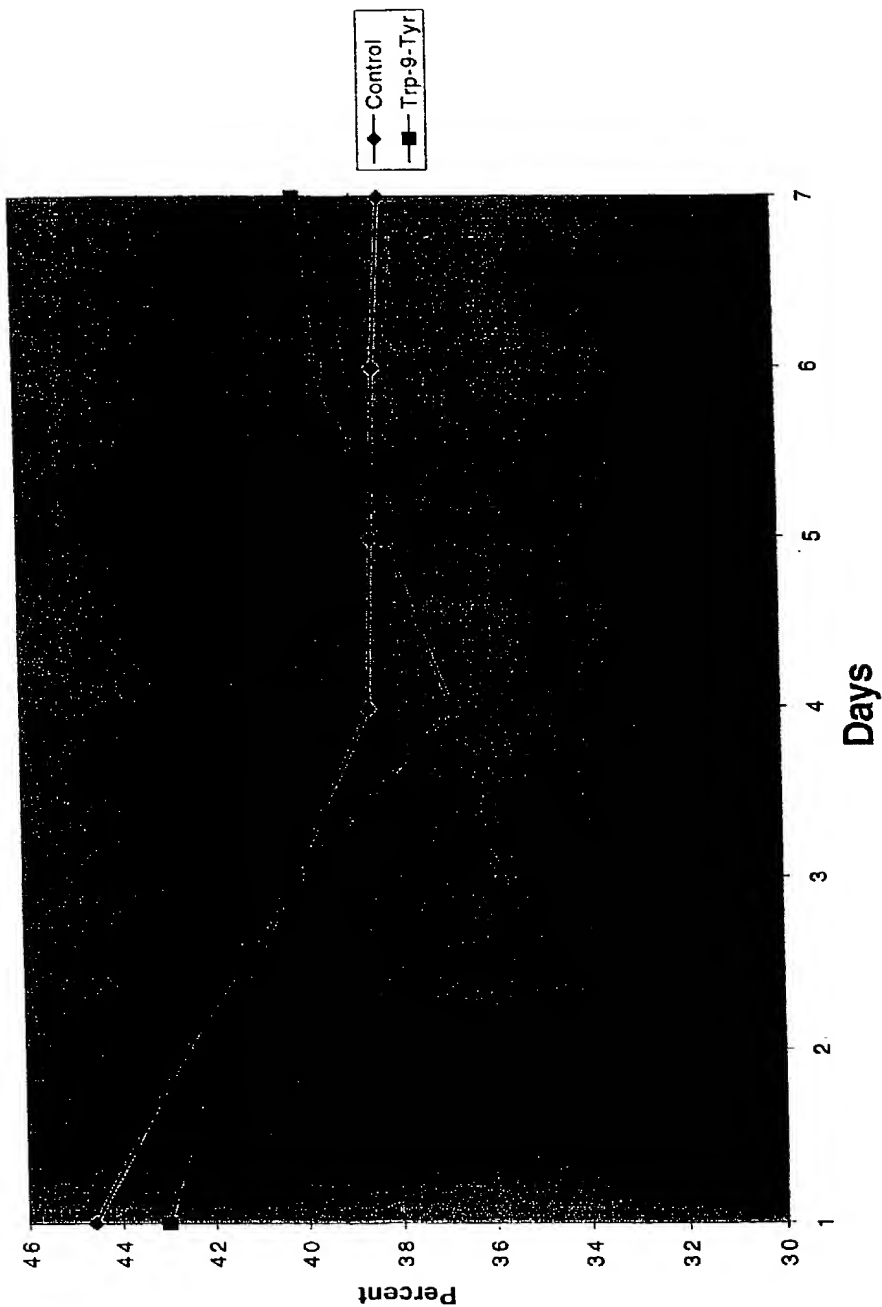


FIG. 7

Trp-9-Tyr (Control)

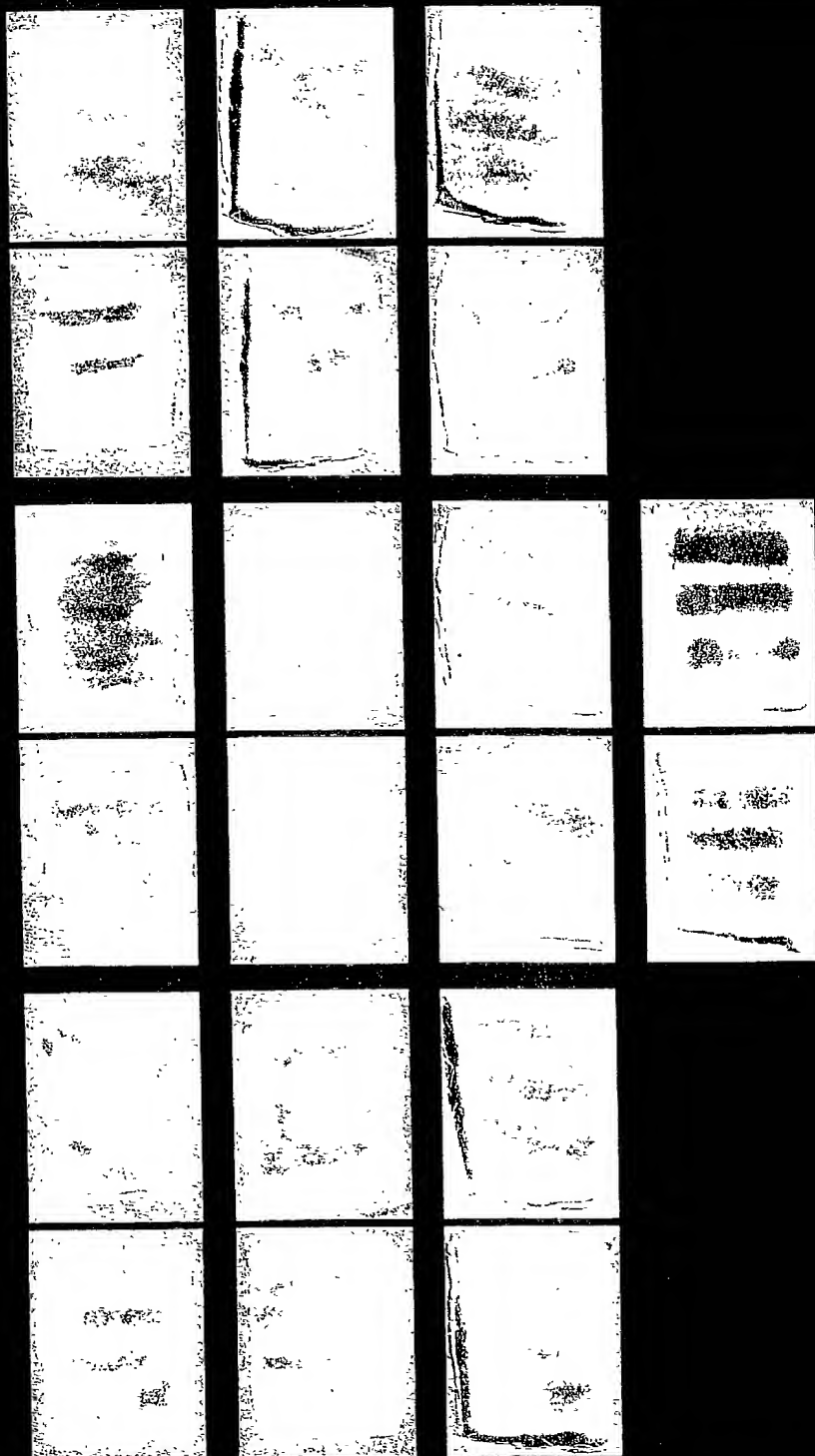


FIG. 8

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Trp-9-Tyr (24-hr Treatment)

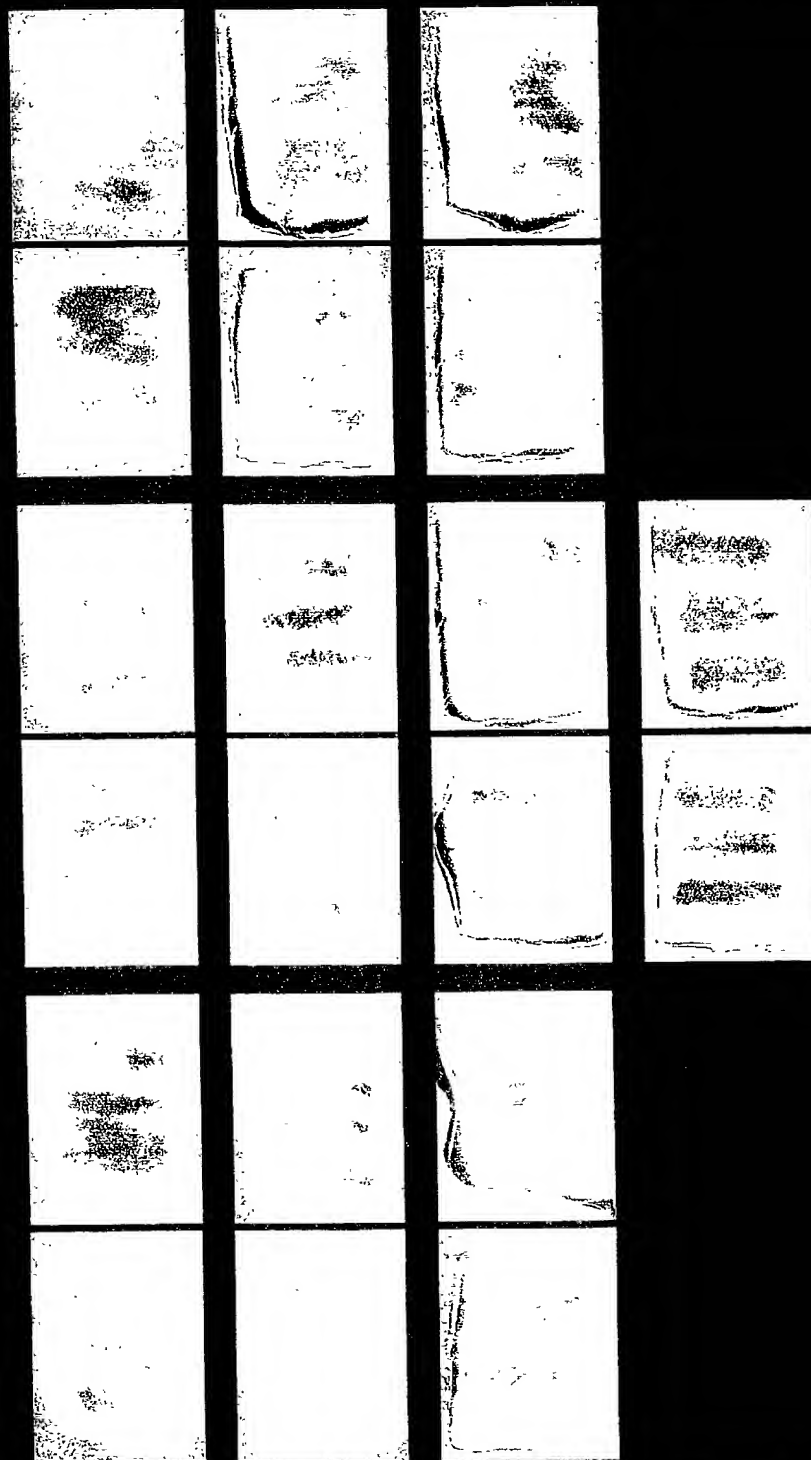


FIG. 9

Trp-9-Tyr (48-hr Treatment)



FIG. 10

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| Abscess Formation at Day 7 | | | | | | | | | |
|----------------------------|---|---|---|---|----|-----|------|--|--|
| Control | 1 | 7 | 8 | 9 | 10 | SUM | Mean | | |
| Rabbit | 2 | 2 | 2 | 2 | 2 | 10 | 2 | | |
| 10 ⁹ | 1 | 0 | 1 | 2 | 1 | 5 | 1 | | |
| 10 ⁸ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 10 ⁷ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 10 ⁶ | | | | | | | | | |
| Trip-9-Tyr | | | | | | | | | |
| Rabbit | 2 | 3 | 4 | 5 | 6 | | | | |
| 10 ⁹ | 2 | 2 | 2 | 2 | 2 | 10 | 2 | | |
| 10 ⁸ | 0 | 1 | 2 | 0 | 1 | 4 | 0.8 | | |
| 10 ⁷ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 10 ⁶ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |

Fig. 11

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| Abscess Size at Day 7 | | (Area=square cm) | | | | | | | |
|-----------------------|-------|------------------|-------|-------|-------|-------|--------|-------|-------|
| Control | 1 | 7 | 8 | 9 | 10 | Mean | sd | SEM | |
| Rabbit | | | | | | | | | |
| 10^9 | 9.00 | 12.71 | 23.00 | 19.74 | 56.16 | 24.12 | 18.747 | 8.407 | |
| 10^9 | 8.10 | 11.52 | 15.84 | 19.76 | 52.00 | 21.44 | 17.638 | 7.910 | |
| 10^8 | 0.25 | 0.00 | 8.00 | 8.91 | 1.70 | 3.77 | 4.336 | 1.944 | |
| 10^8 | 0.00 | 0.00 | 0.00 | 0.80 | 0.00 | 0.16 | 0.358 | 0.160 | |
| Total | | | | | | | | | |
| mean 10^9 | 8.55 | 12.12 | 19.42 | 19.75 | 54.08 | 22.78 | 18.141 | 8.135 | |
| mean 10^8 | 0.13 | 0.00 | 4.00 | 4.86 | 0.85 | 1.97 | 2.290 | 1.027 | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| Trip-9-Tyr | | | | | | | | | |
| Rabbit | | | | | | | | | |
| 10^9 | 13.75 | 11.55 | 20.21 | 17.20 | 16.50 | 15.84 | 3.322 | 1.490 | 0.179 |
| 10^9 | 12.90 | 16.00 | 18.80 | 12.32 | 20.00 | 16.00 | 3.428 | 1.537 | 0.241 |
| 10^8 | 0.00 | 0.35 | 5.20 | 0.00 | 1.40 | 1.39 | 2.206 | 0.989 | 0.119 |
| 10^8 | 0.00 | 0.00 | 2.52 | 0.00 | 0.00 | 0.50 | 1.127 | 0.505 | 0.288 |
| Total | | | | | | | | | |
| mean 10^9 | 13.33 | 13.78 | 19.51 | 14.76 | 18.25 | 15.92 | 2.782 | 1.248 | 0.204 |
| mean 10^8 | 0.00 | 0.18 | 3.86 | 0.00 | 0.70 | 0.95 | 1.653 | 0.741 | 0.174 |

Fig. 12

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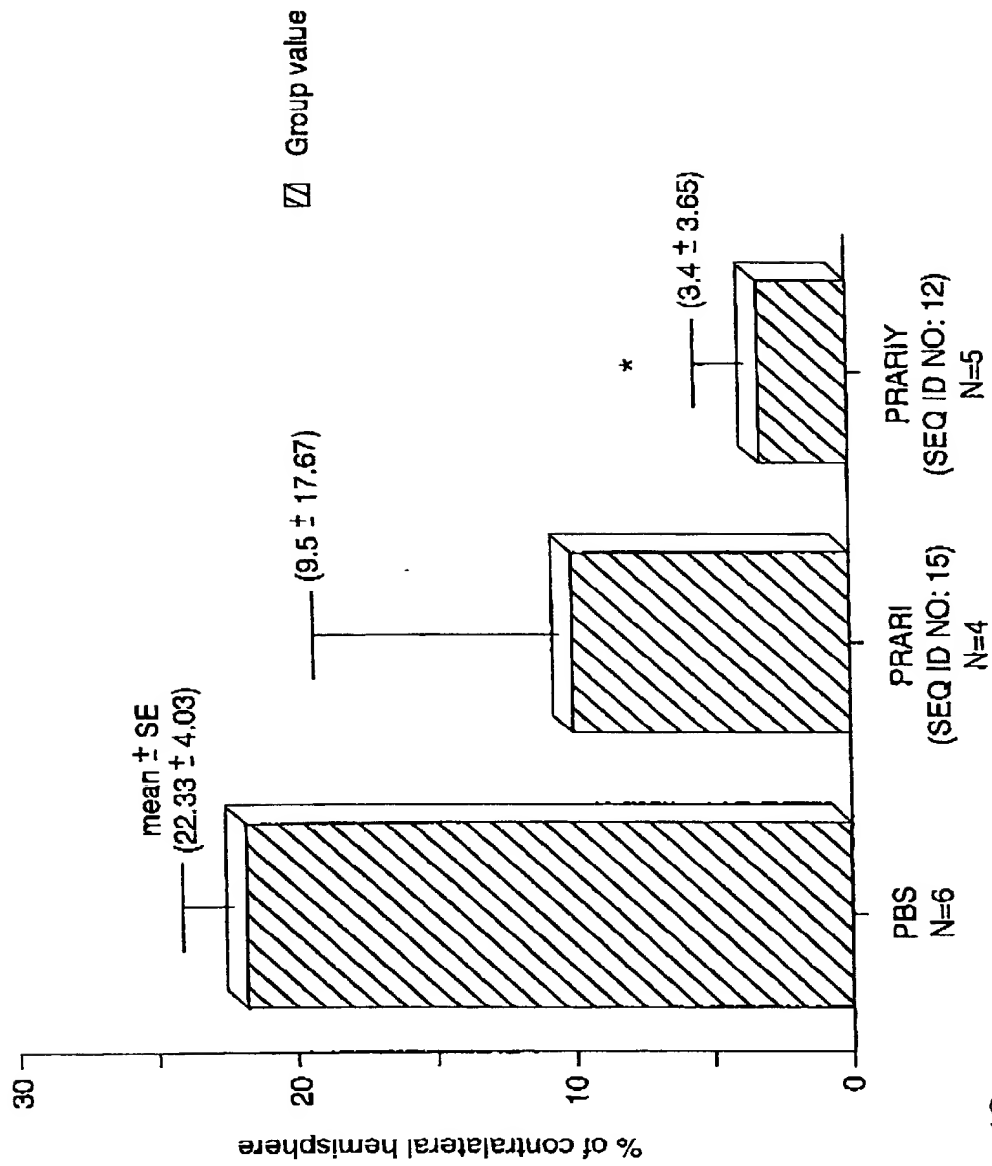


Fig. 13

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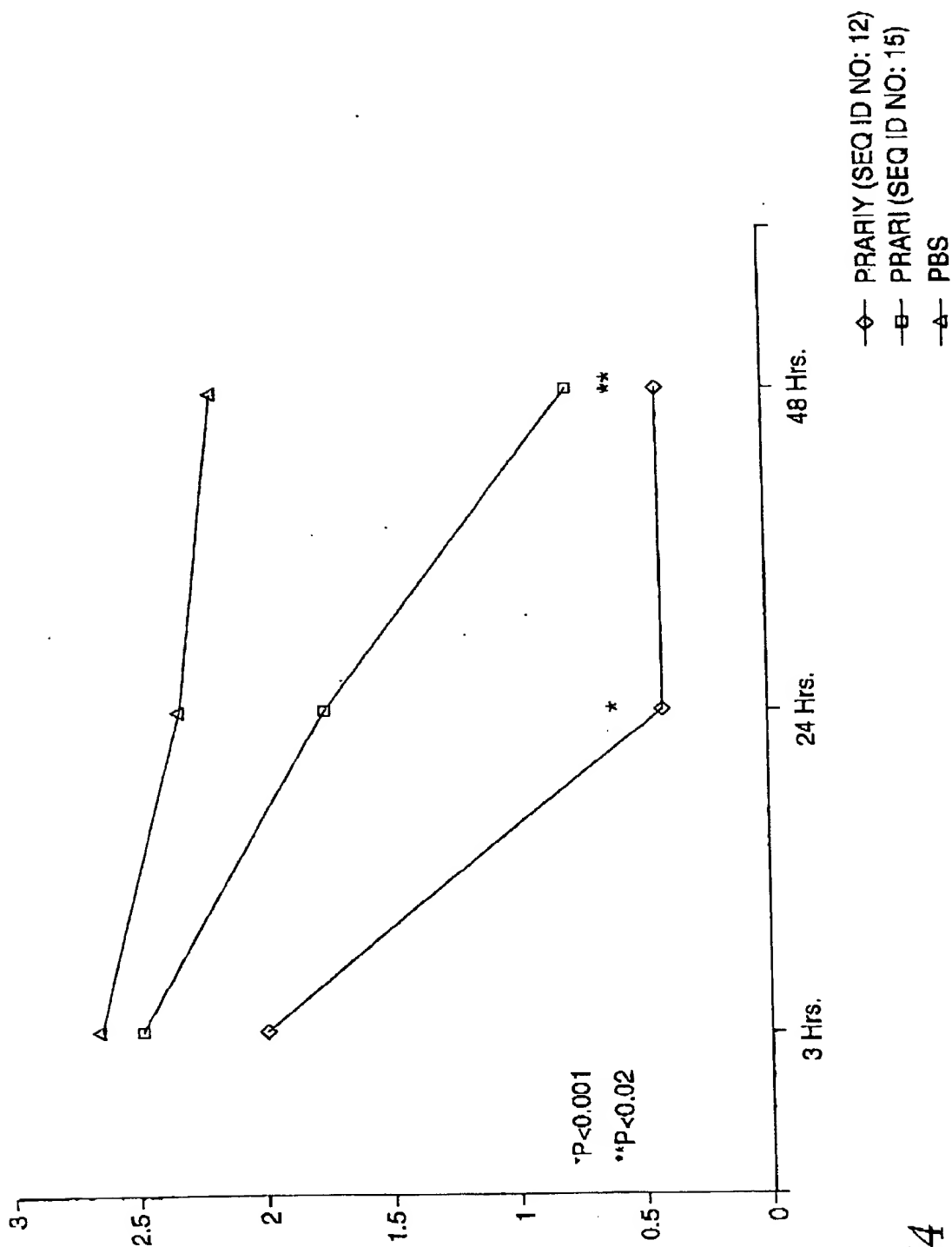


Fig. 14

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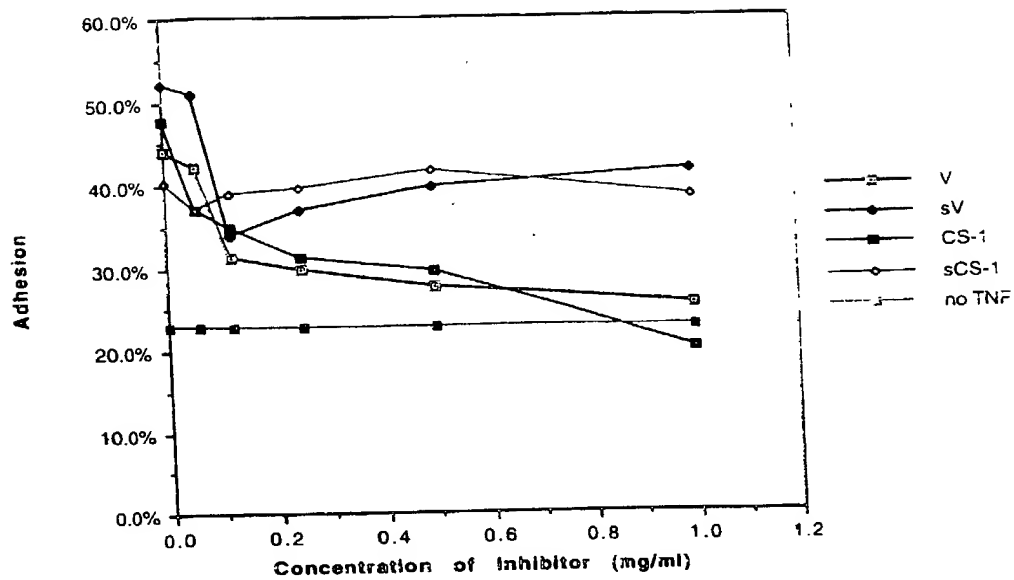


FIG. 15

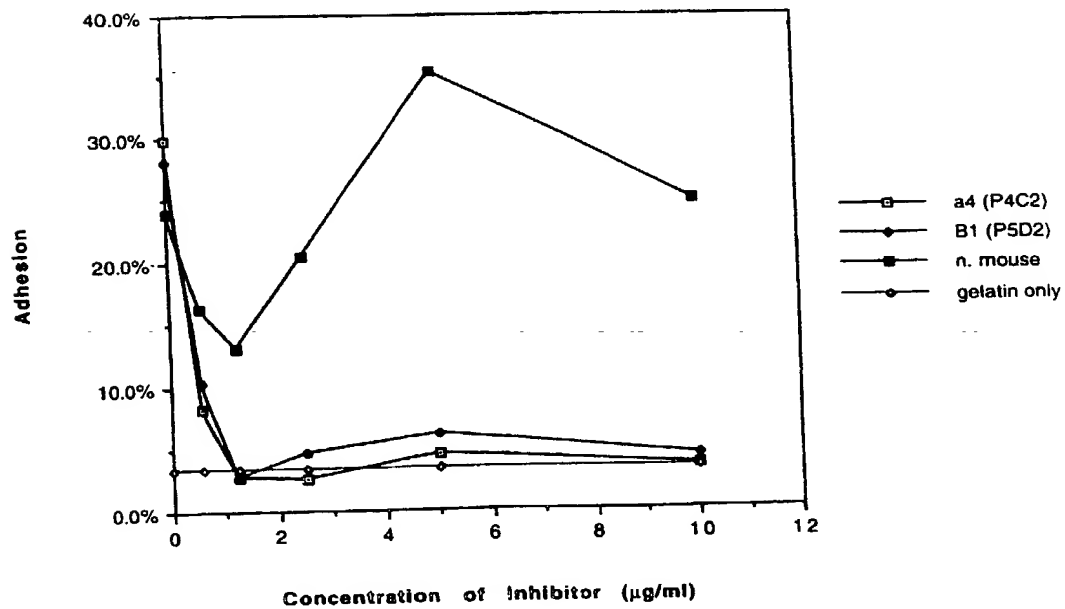
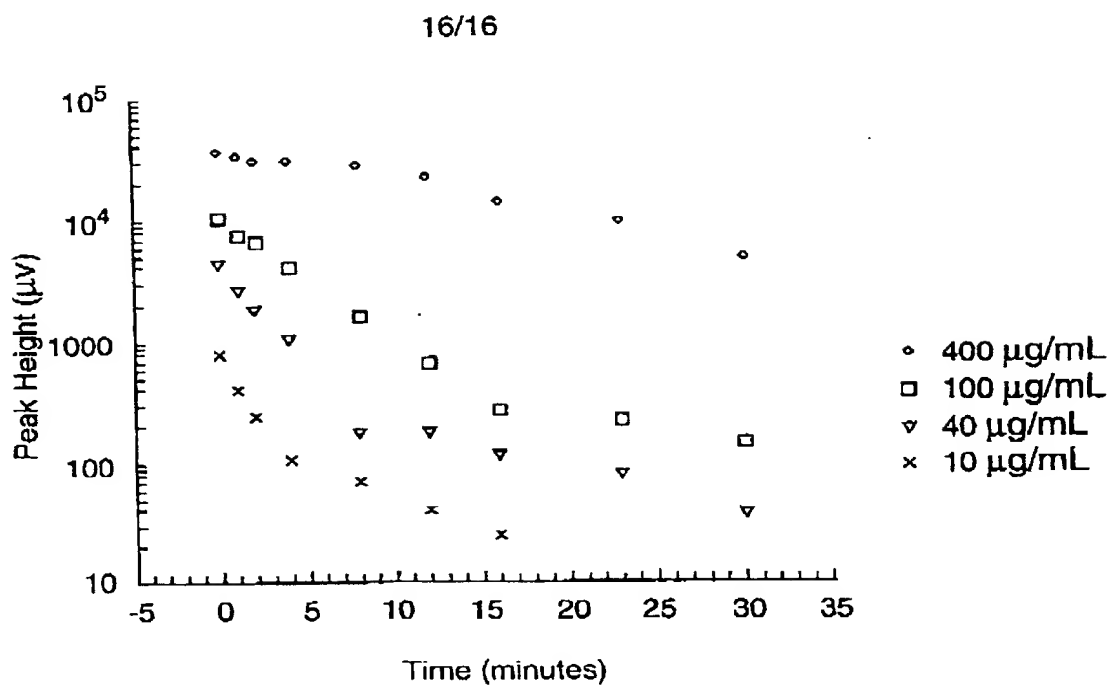
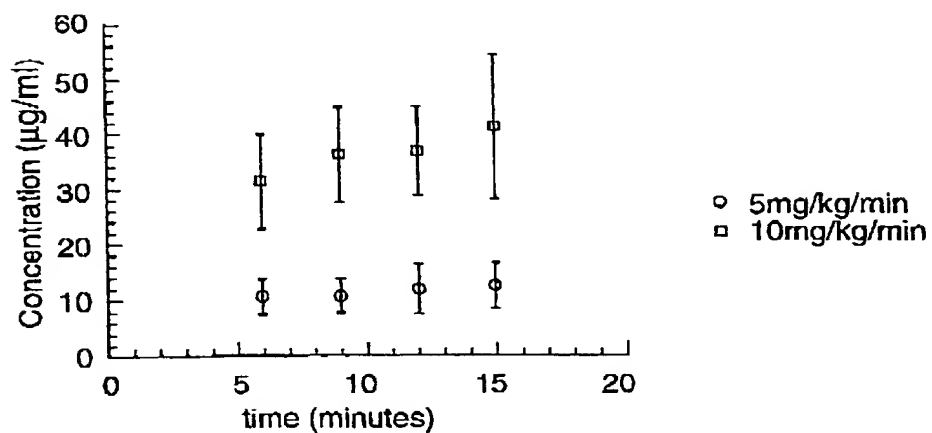


FIG. 16

*Fig. 17**Fig. 18*

DECLARATION AND POWER OF ATTORNEY

We, James B. McCarthy, William J. Mileski, Gordon A. Jamieson, Jr., Walter C. Low, Ronald J. Sawchuk, and Leo T. Furcht, declare that: (1) our respective citizenships and residence/ mailing addresses are indicated below; (2) we have reviewed and understand the contents of the specification identified below, including the claims, as amended by any amendment specifically referred to herein, (3) we believe that we are the original, first, and joint inventors of the subject matter in

METHODS OF USE OF β 1-INTEGRIN INHIBITORS

Filing Date: September 19, 2001

Serial No.: 09/937,076

described and claimed therein and for which a patent is sought; and (4) we hereby acknowledge our duty to disclose to the United States Patent and Trademark Office all information known to us to be material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56.*

We hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below, or §365(a) of any PCT international application which designates at least one country other than the United States of America listed below, and have also identified below any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on the basis of which priority is claimed:

- a. ☒ no such applications have been filed.
 b. ☐ such applications have been filed as follows:

| FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC §119(a)-(d), §365(a), and/or §365(b) | | | |
|--|-------------------------------|--|---|
| COUNTRY | APPLICATION NUMBER | DATE OF FILING (day, month, year) | DATE OF ISSUE (day, month, year) |
| | | | |
| | | | |

| ALL FOREIGN APPLICATIONS, IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S) | | | |
|---|-------------------------------|--|---|
| COUNTRY | APPLICATION NUMBER | DATE OF FILING (day, month, year) | DATE OF ISSUE (day, month, year) |
| | | | |
| | | | |

* Title 37, Code of Federal Regulations, §1.56 is reproduced on the attached page.

Declaration and Power of Attorney

Page 2 of 4

Serial No.: 09/937,076

Filing Date: September 19, 2001 (Int'l Filing Date: March 22, 2000)

Title: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

We hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

- a. no such applications have been filed.
b. X such applications have been filed as follows:

| PROVISIONAL APPLICATION(S), IF ANY, UNDER 35 USC §119(e) | |
|--|--------------------------------------|
| APPLICATION NUMBER | DATE OF FILING (day, month, year) |
| 60/125,634 | 22 March 1999 |
| 60/167,538 | 24 November 1999 |

We hereby claim the benefit under Title 35, United States Code, §120 of any United States applications or §365(c) of any PCT international application(s) designating the United States of America, listed below.

- a. no such applications have been filed.
b. X such applications have been filed as follows:

| APPLICATION NUMBER | DATE OF FILING (day, month, year) | STATUS (patented, pending, abandoned) |
|--------------------|--------------------------------------|--|
| PCT/US00/07680 | 22 March 2000 | Pending |
| | | |

Insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

We hereby appoint Ann M. Muetting (Reg. No. 33,977), Kevin W. Raasch (Reg. No. 35,651), Mark J. Gebhardt (Reg. No. 35,518), Victoria A. Sandberg (Reg. No. 41,287), David L. Provence (Reg. No. 43,022), Matthew W. Adams (Reg. No. 43,459), Loren Albin (Reg. No. 37,763), Kathleen L. Franklin (Reg. No. 47,574), and Joseph C. Huebsch (Reg. No. 42,673) our attorneys with full powers (including the powers of appointment, substitution, and revocation) to prosecute this application and any division, continuation, continuation-in-part, reexamination or reissue thereof, and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence in this case to:

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Minneapolis, MN 55458-1415
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Customer Number 26813

The undersigned declare further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under

Declaration and Power of Attorney

Page 3 of 4

Serial No.: 09/937,076

Filing Date: September 19, 2001 (Int'l Filing Date: March 22, 2000)

Title: METHODS OF USE OF β 1-INTEGRIN INHIBITORS

Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Wherefore, we pray that Letters Patent be granted to us for the invention described and claimed in the specification identified above and we hereby subscribe our names to the foregoing specification and claims, Declaration, and Power of Attorney on the date indicated below.

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Declaration and Power of Attorney

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Serial No.: 09/937,076

Filing Date: September 19, 2001 (Int'l Filing Date: March 22, 2000)

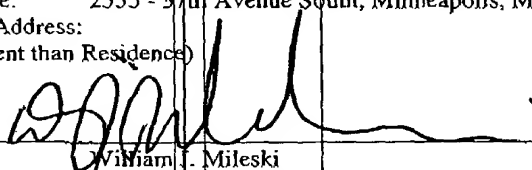
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Serial No. 09/937,076

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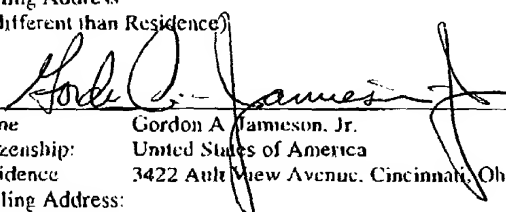
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20 FEBRUARY 2002

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*Declaration and Power of Attorney**Page 3 of 4*

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Mailing Address:
(If different than Residence)

Date

4/24/02

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) Prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) The closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

- (1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or
- (2) It refutes, or is inconsistent with, a position the applicant takes in:
 - (i) Opposing an argument of unpatentability relied on by the Office, or
 - (ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

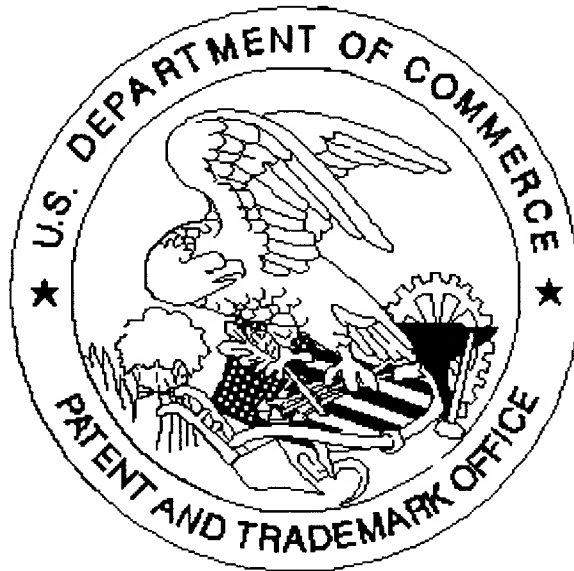
(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

- (1) Each inventor named in the application;
- (2) Each attorney or agent who prepares or prosecutes the application; and
- (3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

(e) In any continuation-in-part application, the duty under this section includes the duty to disclose to the Office all information known to the person to be material to patentability, as defined in paragraph (b) of this section, which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

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Application deficiencies found during scanning:

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